Asian Journal of Andrology (2023) 25, 13–20
www.asiaandro.com; www.ajandrology.com

Open Access

INVITED REVIEW

Reproduction

Generation of male germ cells *in vitro* from the stem cells

Ying-Hong Cui*, Wei Chen*, Si Wu, Cai-Lin Wan, Zuping He

Infertility has become a serious disease since it affects 10%–15% of couples worldwide, and male infertility contributes to about 50% of the cases. Notably, a significant decrease occurs in the newborn population by 7.82 million in 2020 compared to 2016 in China. As such, it is essential to explore the effective methods of obtaining functional male gametes for restoring male fertility. Stem cells, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), spermatogonial stem cells (SSCs), and mesenchymal stem cells (MSCs), possess the abilities of both self-renewal and differentiation into germ cells. Significantly, much progress has recently been achieved in the generation of male germ cells *in vitro* from various kinds of stem cells under the specified conditions, *e.g.*, the coculturing with Sertoli cells, three-dimensional culture system, the addition of growth factors and cytokines, and/or the overexpression of germ cell-related genes. In this review, we address the current advance in the derivation of male germ cells *in vitro* from stem cells based on the studies of the peers and us, and we highlight the perspectives and potential application of stem cell-derived male gametes in reproductive medicine.

Asian Journal of Andrology (2023) **25**, 13–20; doi: 10.4103/aja20226; published online: 15 April 2022

Keywords: application; *in vitro* generation; male germ cells; stem cells

INTRODUCTION

Around 10%–15% of couples are infertile worldwide, and male infertility contributes to about 50% of cases.[1] The Global Burden of Disease Survey reveals that the age-standardized prevalence of infertility was increased annually by 0.291% in men between 1990 and 2017.[2] It has been estimated that there are 50 million of patients with infertility in China, and male factors account for half of these patients. Notably, there are significant decreases in the newborn population by 7.82 million in 2020 (10.04 million) and by 6.07 million in 2019 (11.79 million) compared to 2016 (17.86 million) in China. Continuous production of functional sperm in the testis is the key to male reproduction, and the progression of spermatogenesis starts with the mitotic division of spermatogonial stem cells (SSCs) in puberty and continues throughout adulthood. Problems in any stages of the spermatogenesis, including the mitosis, meiosis, and spermiogenesis, can lead to male infertility. According to the testicular biopsy, male infertility can be divided into two categories, namely obstructive azoospermia (OA) and nonobstructive azoospermia (NOA).[3] Patients with OA have normal spermatogenesis in the testes, and the sperm cannot be detected in the semen due to the obstruction of the sperm transport pipeline. Abnormal spermatogenesis without spermatids exists in the NOA testes. The pathogenesis of NOA is complex, including genetic mutations, chemicals, and endocrine disorders. In addition, many patients with NOA are idiopathic, and their pathogenesis remains largely unclear. Therefore, it is of great significance to explore genetic and epigenetic mechanisms underlying male germ cell development to fully understand the etiology of spermatogenesis failure.

Assisted reproductive technology (ART) has achieved great progress recently, which helps many couples realize their dream of having children. Nevertheless, ART is useful only for men who have mature spermatozoa. In addition, approximately 30% of cancer patients are permanently infertile due to the damage of testis by the chemotherapy or radiotherapy.[4] Moreover, about 46% of male childhood cancer survivors have been reported to be sterile. The most extensively used method for fertility preservation is sperm cryopreservation, but it is invalid for the prepubertal boys who are unable to produce spermatozoa. It is thus essential to develop new strategies of fertility preservation for the patients without active sperm.

With the development of stem cell technology, it becomes feasible to derive male germ cells *in vitro* from various kinds of stem cells. Stem cells, including the embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), SSCs, and mesenchymal stem cells (MSCs), have the capacities of both self-renewal and differentiation. In 2003, Toyooka *et al.*[5] have demonstrated that ESCs can be coaxed to differentiate into primordial germ cells (PGCs), and eventually, they give rise to the spermatids *in vitro*. PGCs can be derived from embryoid bodies, and they generate the functional spermatids that develop into blastocyst when injected into oocytes.[6] These two studies illustrate that the production of sperm *in vitro* might pave the way to an analysis of the mechanism involved in spermatogenesis and sperm maturation and that it offers a novel approach of obtaining male gametes for fertility.

The Key Laboratory of Model Animals and Stem Cell Biology in Hunan Province, Hunan Normal University School of Medicine; The Manufacture-Based Learning and Research Demonstration Center for Human Reproductive Health New Technology of Hunan Normal University, Changsha 410013, China.
*These authors contributed equally to this work.
Correspondence: Dr. Z He (zupinghe@hunnu.edu.cn)
Received: 24 November 2021; Accepted: 22 February 2022

DERIVATION OF MALE GERM CELLS FROM THE SSCS

The PGCs are the precursors that can give rise to the SSCs (also known as male germline stem cells), and they migrate to the mesentery and finally form the testis together with the mesoderm cells of the genital ridge.[7,8] PGCs stay mitotic arrest upon reaching the genital ridges until day 5 postpartum,[9,10] while some of these cells differentiate into SSCs. Spermatogenesis in mammals occurs in the seminiferous epithelium of the testis, and it includes three main stages: the mitosis of SSCs, meiosis of spermatocytes, and maturation of spermatids. As the initial cells of spermatogenesis, SSCs are responsible for maintaining stem cell pool and differentiating into spermatocytes which further give rise to spermatids.

In 1992, Matsui *et al.*[11] and Resnick *et al.*[12] have reported that mouse PGCs can be proliferated and expanded at least 20 passages *in vitro* by the addition of basic fibroblast growth factor (bFGF) and LIF, and octamer-binding protein 4 (Oct4), bone morphogenetic protein 4 (Bmp4), and retinoic acid (RA) are essential for PGC survival.[13–15] However, little is known about the progress of human PGC development. Tilgner *et al.*[16] have enriched SSEA1-positive cells from human ESCs, and SOX17 has been shown to be a key regulator of human PGC fate.[17] It has been reported that mouse PGCs can give rise to functional gametes when they are transplanted with somatic cells under the kidney capsule of adult animals, and these gametes are fertilized to produce normal offspring.[18] Nevertheless, there are few studies showing the differentiation of PGCs into male sperm *in vitro*. Nakatsuji and Chuma[19] developed a two-dimensional culture system with mixing PGCs with endogenous somatic cells and making KL-producing cell monolayer as feeder cells, which induced the differentiation of PGCs into meiotic prophase I cells. Recently, Yuan *et al.*[20] have established a new culture system for human testicle organogenesis *in vitro* from the fetal gonadal ridge. The gonad tissue fragments were cultured on small agarose gels semi-submerged in the medium using a standard gas–liquid interphase method.[20] Notably, complete process of spermatogenesis, including the mitosis of spermatogonia, meiosis of spermatocytes, and spermiogenesis of spermatids to form functional sperm,[20] has been detected in human testicle organogenesis *in vitro*. Significantly, the spermatids produced by this testicle organogenesis possess the normal methylation status of imprinted genes, which implicates the abilities of fertilization and early embryonic development *in vitro*[20] (**Figure 1**).

Compared with PGCs, different culture systems of SSCs have been established for their survival and the generation of the differentiated male germ cells. Kubota *et al.*[21] have demonstrated that mouse SSCs can be cultured with serum-free medium containing glial cell line-derived neurotrophic factor (GDNF), bFGF, and GFRA1, which promotes SSCs to proliferate and survive for over 6 months. Long-term culture of human SSCs has been achieved by Sadri-Ardekani and his colleagues.[22] We have revealed that a number of epigenetic and genetic factors stimulate the proliferation of human SSCs and inhibit their apoptosis. We have demonstrated that several microRNAs, including miR-1908-3p, miR-663a, miR-31-5p, and miR-122-5p, mediate the proliferation and apoptosis of human SSCs.[23–26] We have also shown that *PAK1* and *FGF5* genes are involved in the regulation of human SSC development.[27,28] These studies indicate the possibility for the expansion and differentiation of SSCs into sperm *in vitro* (**Figure 1**).

Several induction systems of SSC differentiation have been established. In 2002, Feng *et al.*[29] established an immortalized cell line using the undifferentiated type A spermatogonia from 6-day-old mice by the overexpression of TERT, and the mouse spermatogonial cell line can be induced by stem cell factor (SCF) to differentiate into spermatocytes and the haploid cells. Wang and his colleagues isolated and purified murine SSCs, and RA was used to induce the differentiation of murine SSCs into haploid germ cells.[30] A two-dimensional culture system has been developed for the proliferation and differentiation of mouse SSCs, and RA promotes the production of haploid germ cells derived from SSCs *in vitro*.[31] Nevertheless, the fertilization ability of the sperm derived from mouse SSCs remains unknown. In 2011, Sato's team has transplanted the mouse SSCs into the seminiferous tubules, and they cultured the host testis fragments in 1.5% agarose gel and α-MEM supplemented with 10% knockout serum replacement. After 43 days of culture, round spermatids can be collected, and these haploid cells can produce the pups after ROSI with oocytes.[32] Notably, we have established a three-dimensional-induced system that can coax human SSCs to differentiate into functional sperm *in vitro*.[33] Specifically, we cocultured human SSCs with the inactivated human Sertoli cells in a ratio of 1:3, and these cells were cultured in matrigel with the conditioned medium.[33] Compared with the two-dimensional culture system, the percentages of haploid cells are obviously higher in three-dimensional-induced system.[33] The *in vitro* differentiation of SSCs into functional sperm in rodents has been developed, and healthy offspring and second generation can be delivered by the *in vitro*-derived germ cells. Despite the generation of sperm from human SSCs, there are certain issues to be defined. For example, the number of human primary SSCs is very limited, and thus, the expansion of human SSCs *in vitro* is required to obtain sufficient cells. In addition, the concerns regarding the differentiation conditions *in vitro*, the efficiency, the

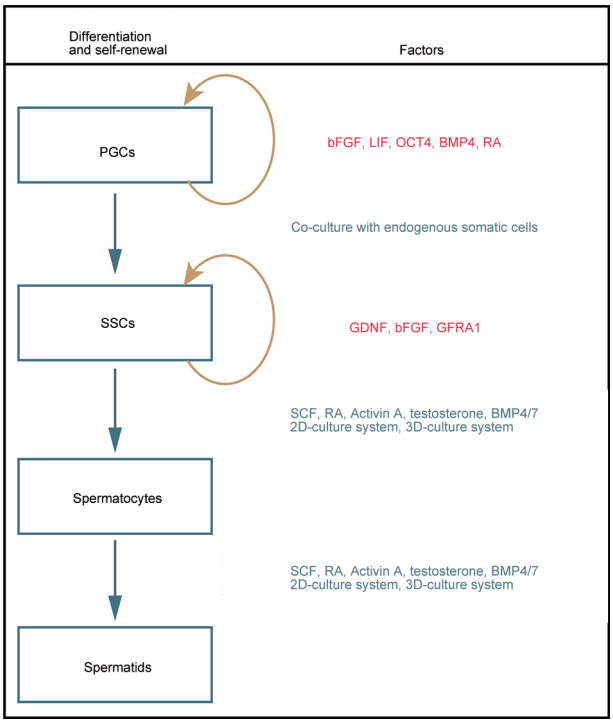

Figure 1: Schematic diagram implicates the derivation of male germ cells from SSCs *in vitro*. The right panel includes the related factors for the generation of male germ cells *in vitro* from the SSCs. The red indicates the genes involved in the self-renewal, while the blue denotes the genes related to the differentiation of germ cells. SSCs: spermatogonial stem cells; PGCs: primordial germ cells; bFGF: basic fibroblast growth factor; LIF: leukemia inhibitory factor; OCT4: octamer-binding protein 4; BMP4: bone morphogenetic protein 4; RA: retinoic acid; SCF: stem cell factor.

safety of *in vitro*-produced spermatids, and the ethical issues remain to be resolved.

DERIVATION OF MALE GERM CELLS FROM THE ESCS

ESCs are derived from early embryos or primitive gonads. The main characteristics of ESCs are their pluripotency and differentiation into all cell types in the body.[34,35] As such, it would be feasible to induce ESCs to differentiate into functional spermatids (**Table 1**). In 2003, ESCs have been shown to differentiate into PGCs by coculturing with trophoblast cells or the BMP4-producing cells. Eight weeks after the transplantation of ESCs-derived PGCs and gonadal cells under a host testis capsule, testicular tubules and spermatids are observed.[5] Furthermore, Geijsen *et al*.[6] have selected SSEA1$^+$ cells from embryoid bodies (EBs) and induced the EB differentiation into round spermatids that are able to fertilize with oocytes and develop into the two-cell embryo and blastocyst. These studies suggest that ESCs can form functional haploid male gametes. Subsequently, Clark *et al*.[36] reveal the transcriptional and translational profiles during ESC differentiation into spermatids. Low glucose medium suppresses germ cell formation from ESCs.[37] The Stra8-EGFP and Prm1-DsRed promoters have been utilized to isolate spermatocytes and spermatids from the ESCs *in vitro*,[38] respectively, and notably, these spermatids can fertilize with mouse oocytes to produce healthy offspring.[38] Monkey ESCs can also be induced to differentiate into male germ cells by detecting the germ cell markers,[39] while mouse ESCs give rise to male germ cells by the overexpression of DAZL.[40] Complete meiosis of ESCs has been achieved by coculturing ESCs-derived PGCs with postnatal testicular cells and the addition of Activin A, BMPs, and RA,[41] or by the overexpression of Eif2s3y.[42] Human ESCs have been shown to be coaxed by the conditioned medium with the addition of RA and BMP4 to generate spermatids *in vitro*.[43] Interestingly, coculturing human ESCs with mitomycin-C inactivated porcine ovarian fibroblasts has been found to be an appropriate condition for the differentiation of human ESCs into male germ cells *in vitro*,[44] while Activin A promotes the differentiation potential of human ESCs into germ cells.[45]

Many factors affect the efficiency of male germ cells from ESCs. KIT ligand and BMP signaling enhance the differentiation of human ESCs into germ cells.[46] The loss of KITL causes a significant decrease in the enrichment of human germ cells.[46] BMP4 increases the levels of germ cell markers and appears to promote the differentiation of human ESCs into germ cells.[46] MiR-34c has been shown to mediate mouse ESC differentiation through RARg,[47] and hedgehog and JAK-STAT signaling pathways are involved in the regulation of chicken ESC differentiation into male germ cells.[48,49] Nevertheless, the molecular

Table 1: The embryonic stem cell differentiation into male germ cells *in vitro*

Sources of the ESCs	*Methods*	*Outcomes*	*Functional assays*	*References*
MVH-positive mouse ESCs	ESCs are cocultured with trophoblast cells or BMP4-producing cells	Testicular tubules and spermatids are detected	Not determined	5
Mouse ESCs	Conditioned-medium contains 15% IFS, 200 μg ml^{-1} iron-saturated transferrin, 4.5 mmol l^{-1} monothiolglycerol, 50 μg ml^{-1} ascorbic acid, and 2 mmol l^{-1} glutamine	Round spermatids are observed	Half the injected oocytes with round spermatids form 2-cell embryo, and 20% of them develop into blastocysts	6
LacZ-positive mouse ESCs	ESCs are cocultured with trophoblast cells or the BMP4-producing cells in a LIF-free medium containing glucose	Low glucose medium suppresses germ cell formation	Not determined	37
Mouse ESC cell line	*Stra8-EGFP* and *Prm1-DsRed* are utilized for selecting spermatocytes and spermatids from ESCs to form SSCs, and RA is used to induce meiosis *in vitro*	Functional haploid male gametes are formed and health offspring are delivered	Male gametes can be fertilized and produce offspring	38
Monkey ESC cell line	Mouse inactivated embryonic fibroblasts are used as the feeder cells, and Ham's nutrient mixture F-12 supplemented with 0.1 mmol l^{-1} 2-mercaptoethanol, 1000 U ml^{-1} LIF, and 15% FBS served as the conditioned medium	Male germ cells are formed	Not determined	39
Mouse ESC cell line	Overexpression of Dazl and DMEM/F-12 medium containing 15% FBS are employed to induce spermatogenesis	Motile spermatids are detected	Not determined	40
Mouse ESCs	PGCs from ESCs are cocultured with postnatal testicular cells in medium supplemented with Activin A, BMPs and RA	Functional haploid male gametes are formed and health offspring are delivered	Male gametes can be fertilized and produce offspring	41
Mouse ESCs	Mouse embryonic fibroblasts are used as the feeder layer in the conditioned-medium containing 50 ng ml^{-1} BMP4, 50 ng ml^{-1} SCF, 50 ng ml^{-1} Bmp8a, 12 μg ml^{-1} insulin, and 10% KSR For SSC differentiation, culture medium comprises 25 ng ml^{-1} BMP4, 20 ng ml^{-1} bFGF, 40 ng ml^{-1} GDNF, 10 ng ml^{-1} LIF, 12 μg ml^{-1} insulin, and 15% KSR	Haploid cells are generated from ESCs *in vitro*	Spermatids from ESCs can be fertilized	42
Human ESC line	Three different induction conditions are used as follows: (i) EB medium supplemented with 10 ng ml^{-1} BMP4; (ii) EB medium supplemented with 2 mmol l^{-1} RA; and (iii) conditioned medium from neonatal mouse testis	Postmeiotic spermatids are detected	Not determined	43
Human ESCs	The conditioned medium is composed of KO-DMEM, 20% FBS, 1% NEAA, 1% L-glutamine, 0.1 mmol l^{-1} β-mercaptoethanol, 50 ng ml^{-1} BMP4, and 20 ng ml^{-1} Activin A	The expression of germ cell markers, including VASA, is detected	Not determined	45

BMP8a: bone morphogenetic protein 8a; ESCs: embryonic stem cells; MVH: mouse vasa homolog; BMP4: bone morphogenetic protein 4; RA: retinoic acid; FBS: fetal bovine serum; PGCs: primordial germ cells; SCF: stem cell factor; KSR: knockout serum replacement; SSCs: spermatogonial stem cells; bFGF: basic fibroblast growth factor; GDNF: glial cell line-derived neurotrophic factor; EB: embryoid body; KO-DMEM: knockout Dulbecco's Modified Eagle's Medium; IFS: inactivated fetal bovine serum; LIF: leukemia inhibitory factor; NEAA: nonessential amino acids

mechanisms underlying human ESC differentiation into male germ cells remain largely unclear, and the effective methods to generate male germ cells from human ESCs need to be further explored.

DERIVATION OF MALE GERM CELLS FROM THE IPSCS

In 2006, Takahashi and Yamanaka[50] have for the first time generated the iPSCs from mouse fibroblasts via the overexpression of four transcription factors, namely Oct3/4, SRY-Box transcription factor 2 (Sox2), c-Myc and Kruppel-like factor 4 (Klf4), as evidenced the findings that these cells are similar to ESCs in morphology, gene and protein expression profiles, epigenetic modification status, cell proliferation ability, embryoid body, teratoma formation ability, and differentiation ability. In human, fibroblasts can be reprogrammed to become the iPSCs which are almost identical to ESCs by the four defined factors, *e.g.*, Oct3/4, Sox2, Klf4, and c-Myc (method of Shinya Yamanaka laboratory) or Oct3/4, Sox2, NANOG, and LIN28

Table 2: The induced pluripotent stem cell differentiation into male germ cells *in vitro*

Sources of the iPSCs	*Methods*	*Differentiation methods*	*Outcomes*	*Functional assays*	*References*
Mouse hepatocytes-derived iPSCs	Overexpression of Oct4, Sox2 and Klf4	iPSCs are cocultured with M15-BMP4 cells and with the addition of GDNF and EGF	Male germ cell markers are detected	Not determined	53
Mouse iPSC line	Unavailable	Induction of iPSCs into EBs is performed in LIF-free medium and differentiation of iPSCs-derived EBs into SSCs is achieved by RA treatment	SSCs are observed	Not determined	54
miPSC-4.1 line	Unavailable	Induction of iPSCs into EBs is conducted by hanging drop method in LIF-free medium; for the differentiation, 7-day EBs are cultured on the inactivated MEFs in medium composed of 15 ng ml^{-1} bFGF, 30 ng ml^{-1} SCF and 2 μmol l^{-1} all-trans RA	SSCs are detected	Not determined	56
Mouse iPSC line	Unavailable	The iPSCs are cultured with DMEM supplemented with 15% FBS, 1 mmol l^{-1} L-glutamine, 1% nonessential amino acids, and 0.1 mmol l^{-1} β-mercaptoethanol to form EB that are induced with 0.1 mmol l^{-1} RA or 10% porcine follicular fluid in spontaneous differentiation medium	Male germ cells are formed	Not determined	57
Mouse iPSC line Tg-GFP-miPSC11.1 line	Unavailable	Induction of iPSCs into EBs has be done by hanging drop method in LIF-free medium; for the differentiation, 2 mol l^{-1} RA or 1 mol l^{-1} testosterone or 2 mol l^{-1} RA and 1 mol l^{-1} testosterone combination is utilized	Male germ cells can be formed	Not determined	58
Adult tail-tip fibroblasts-derived iPSCs	Fibroblasts are transfected with the pMXs-mOct4, pMXs-mSox2, or the pMXs-mKlf4, pMXs-mc-Myc	For iEpiLC induction, iPSCs are treated with 20 ng ml^{-1} Activin A and 5 ng ml^{-1} bFGF combined with 1% SR in differentiation medium (GMEM with N2, B27, 0.1 mmol l^{-1} NEAA, 1 mmol l^{-1} sodium pyruvate, 0.1 $mmol^{-1}$ 2-mercaptoethanol, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin and 2 mmol l^{-1} l-glutamine). For iPGCLC differentiation, iEpiLCs are induced with 50 ng ml^{-1} BMP4, 50 ng ml^{-1} BMP8b, and 50 ng ml^{-1} SCF in differentiation medium containing 10% SR	SSCs are induced	Not determined	59
Porcine embryonic fibroblasts- derived iPSCs	Not mentioned	For the EpiLCs differentiation, piPSCs are cultured in N2B27 medium including 20 ng ml^{-1} Activin A, 12 ng ml^{-1} bFGF and 1% KSR; for the PGCLC differentiation, EpiLCs are treated with 50 ng ml^{-1} BMP4, 50 ng ml^{-1} BMP8B, 50 ng ml^{-1} SCF, 50 ng ml^{-1} EGF, and 1000 U ml^{-1} LIF in GMEM with 15% KSR, NEAA, L-glutamine, and β-mercaptoethanol; for the SSCLCs differentiation, PGCLCs or EpiLCs are cultured with DMEM medium containing 15% FBS, NEAA, L-glutamine, β-mercaptoethanol, and cytokines, including 2 μmol l^{-1} RA and 4 ng ml^{-1} GDNF, and 1 μmol l^{-1} testosterone	SSCLC and the meiosis can be detected	Not determined	62
Human CBiPSC and KiPSC lines	Unavailable	iPSCs are cocultured with human foreskin fibroblasts in human ESC medium with the KO-DMEM containing 20% KO serum replacement, nonessential amino acids (1x) and GlutaMAX (1x) for 3 weeks. RA is used for 3 further weeks. After 6 weeks of differentiation, all cells are cultured in presence of LIF, bFGF, FRSK, and CYP26 inhibitor for 4 more weeks	Spermatogonia-like cells, complete meiosis, and haploid cells are observed	Not determined	63
Human foreskin fibroblast cells-derived iPSCs	Fibroblasts are overexpressed with *Oct3/4*, *Sox2*, *c-Myc* and *Klf4*	For the EBs induction, iPSCs are cultured with DMEM supplemented with 10% KSR, 5% FBS, 2 mmol l^{-1} L-glutamine, 0.1 mmol l^{-1} NEAA, 0.5 mmol l^{-1} ascorbic acid, 10 mg ml^{-1} ITS, 0.1 mmol l^{-1} β-mercaptoethanol, 100 ng ml^{-1} BMP4, and 0.1 mmol l^{-1} RA For the differentiation, EBs are cultured on either gelatin-coated plates or DAM scaffolds as 2D and 3D culture, respectively. After 3 days, 200 ng ml^{-1} FSH, 50 mg ml^{-1} BPE, 10 mmol l^{-1} testosterone, 10 ng ml^{-1} bFGF, and 20 ng ml^{-1} EGF are added to the medium and the cells are incubated for an additional 2 weeks	Haploid cells are detected	Not determined	64

iPSCs: induced pluripotent stem cells; Oct4: octamer-binding protein 4; Sox2: SRY-Box transcription factor 2; Klf4: Kruppel-like factor 4; BMP4: bone morphogenetic protein 4; GDNF: glial cell line-derived neurotrophic factor; EGF: epidermal growth factor; EB: embryoid body; SSCs: spermatogonial stem cells; RA: retinoic acid; MEF: mouse embryo fibroblast; bFGF: basic fibroblast growth factor; SCF: stem cell factor; FBS: fetal bovine serum; GMEM: Glasgow's minimum essential medium; NEAA: nonessential amino acids; iPGCLCs: induced PGC-like cells; iEpiLCs: induced epiblast-like cells; EpiLCs: epiblast-like cells; piPSCs: porcine-induced PSCs; KSR: knockout serum replacement; PGCLCs: PGC-like cells; SSCLCs: spermatogonial stem cell-like cells; ESCs: embryonic stem cells; FRSK: forskolin; CBiPSC: cord blood-derived iPSCs; KiPSC: keratinocyte-derived iPSC; BMP8b: bone morphogenetic protein 8b; DAM: decellularized amnion membrane; FSH: follicle-stimulating hormone; BPE: bovine pituitary extract; KO-DMEM: knockout Dulbecco's Modified Eagle's Medium; LIF: leukemia inhibitory factor

(method of Thompson laboratory), respectively.[51,52] Significantly, the iPSCs can be induced to differentiate into cell lineages of the three germ layers, including male germ cells (**Table 2**). The pluripotency of the iPSCs offers a novel application for generating male gametes in regenerative medicine without ethics issue compared to the human ESCs.

In 2010, the differentiation of iPSCs into male germ cells *in vitro* has been achieved via the coculture with M15-BMP4 cells transduced with *GDNF* and epidermal growth factor (*EGF*).[53] In 2012, Zhu *et al*.[54] induce the iPSCs into EBs using the LIF-free medium and differentiate the iPSCs-derived EBs into SSCs by RA, and EBs-derived SSCs undergo spermatogenesis and produce round spermatids when transplanted into mouse testes. The iPSC-derived EBs are grafted with testicular cells into the dorsal skin of mice and reconstitute the seminiferous tubules.[55] Subsequently, several groups have demonstrated the feasibility of inducing mouse iPSCs to differentiate into spermatids *in vitro*,[56–59] while RA and 17β-estradiol contribute to the generation of male germ cells from mouse iPSCs.[60,61] In addition, porcine iPSCs can be produced from embryonic fibroblasts, and they could be induced to differentiate into SSCs.[62]

For human iPSCs, Eguizabal *et al*.[63] have obtained the postmeiotic cells from human iPSCs via the RA-conditioned medium, and spermatogonia, spermatocytes, and haploid spermatids can be generated in the presence of LIF, bFGF, forskolin (FRSK), and CYP26 inhibitor.[63] Notably, 2.3% of haploid cells can be obtained from human iPSCs. In addition, three-dimensional decellularized amnion membrane scaffold has been shown to enhance the differentiation efficiency with 3.95% of human iPSCs into haploid spermatids compared to the 2.22% of haploid cell formation by the two-dimensional culture system.[64] On the other hand, not all iPSCs have the potential to produce male germ cells, and the function of spermatids derived from the iPSCs requires further studies. Compared with ESCs, the iPSCs have the advantage of overcoming the ethical restriction for their application in reproductive medicine. Another constrain is the genetic heterogeneity of the iPSCs and the variability in the differentiated cell phenotype and gene expression.

DERIVATION OF MALE GERM CELLS FROM THE MSCS

MSCs are multipotent stem cells with high self-renewal and differentiation abilities, and they can be obtained from the umbilical cord, bone marrow, peripheral blood vessels, and fat. Like the ESCs and other adult stem cells, they can be cultured and expanded *in vitro* and can also differentiate into a number of cell types, including nerve cells, osteoblasts, chondrocytes, muscle cells, and fats cells, under the specific conditions. One important property of MSCs is the immune suppressive, which is helpful for the application of MSCs in regenerative medicine and reproductive medicine. Here, we summarized the ability of MSCs with differentiating into germ cells *in vitro* (**Table 3**).

Human Wharton's Jelly-derived MSCs have been shown to become germ cells *in vitro* when they are cultured with testicular cells-conditioned medium with the addition of RA and testosterone,[65] as evidenced by the expression of germ cell markers, including Kit, CD49f, Stella, and Vasa.[65] Human adipose-derived MSCs can also be induced into germ-like cells by RA.[66] RA is an important regulator of meiosis in gametogenesis, and it induces the expression of meiosis markers during spermatogenesis.[67] Growth factors, *e.g.*, LIF, GDNF, and EGF, contribute to the long-term culture of MSCs, whereas RA promotes germ cell differentiation of MSCs.[68,69]

Sertoli cells are the only somatic cells within the seminiferous tubules, and they provide an essential microenvironment or the niche for germ cell development. Human MSCs are cocultured with mouse Sertoli cells to induce their differentiation into germ cells since STELLA, VASA, and DAZL are detected in these cells.[70] This finding is consistent with the observations by Segunda *et al*.[71] that coculturing bovine fetal MSC with Sertoli cells leads to male germ cell formation. Coculture with mouse testicular cells promotes the differentiation of

Table 3: The mesenchymal stem cell differentiation into male germ cells *in vitro*

Sources of the MSCs	*Methods*	*Outcomes*	*Functional assays*	*References*
Human MSCs	H-DMEM supplemented with 5% FBS, 50% filtered testicular-cell-conditioned medium, 2 mmol l^{-1} all-trans RA and 1 mmol l^{-1} testosterone are used	Germ cell markers are detected	Not determined	65
Human MSCs, bovine fetal MSCs and human umbilical cord MSCs	MSCs are cocultured with Sertoli cells	Germ cell markers are detected	Not determined	70–72
Human adipose-derived MSCs	MSCs are cultured in H-DMEM complete medium containing 10 μmol l^{-1} RA	Germ cell-specific markers are detected	Not determined	66
Human umbilical MSCs	The 50% Sertoli cell-conditioned medium, 40% DMEM, 10% FBS and RA are employed	Germ cell-specific markers can be detected	Not determined	73
Adipose-derived MSCs	MSCs are culture with testosterone and Sertoli cells	Germ cell-specific markers are detected	Not determined	77
Wharton's jelly-derived MSCs	Complete medium supplemented with 2 mmol l^{-1} L-glutamine and 10 μmol l^{-1} all-trans RA is used for 14 days, and Sertoli cell-conditioned medium has been employed for 3 weeks	Elongating spermatids are observed	Not determined	74
BM-MSCs	MSCs are cultured with the complete medium with 100 ng ml^{-1} BMP4, 100 ng ml^{-1} BMP8b or 10 ng ml^{-1} TGFβ1	SSC and spermatogonium-like markers are detected	Not determined	80
Mouse amniotic MSCs	MSCs are treated with 25 ng ml^{-1} BMP4 for 5 days and then cultured with 1 μmol l^{-1} RA for 12 days	Germ cell markers are detected	Not determined	78
Canine adipose MSCs	MSCs are cultured with 12.5 ng ml^{-1} BMP4	Germ cell markers are detected	Not determined	81
Mouse bone marrow-derived MSCs	MSCs are cocultured with mouse testicular cells in the presence of BMP4	Germ cell markers are detected	Not determined	82
Goat bone marrow MSCs	MSCs are overexpressed with BOULE, DAZL, and STRA8	Germ cell markers can be detected	Not determined	84

MSC: mesenchymal stem cell; FBS: fetal bovine serum; RA: retinoic acid; BMP4: bone morphogenetic protein 4; BMP8b: bone morphogenetic protein 8b; TGFβ1: transforming growth factor beta1; SSCs: spermatogonial stem cells; BM-MSCs: bone marrow MSCs; H-DMEM: dulbecco's modified eagle medium with high glucose

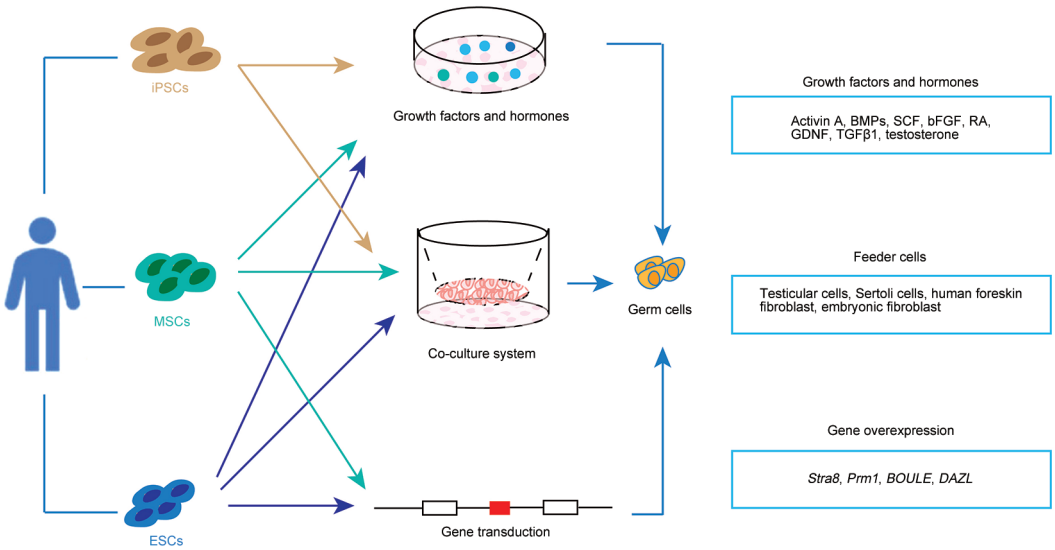

Figure 2: Schematic diagram shows the male germ cells from various types of stem cells *in vitro*. iPSCs: induced pluripotent stem cells; MSCs: mesenchymal stem cells; ESCs: embryonic stem cells.

human MSCs from the umbilical cord into germ-like cells.[72] These studies illustrate that Sertoli cells and the testicular cells may provide the niche, which facilitates the differentiation of MSCs into male germ cells. The combination of RA with Sertoli cells-conditioned medium is employed as the stimulator to evaluate the MSC differentiation ability,[73–76] while RA and testosterone have been shown to enhance male germ-like cell formation from adipose-derived MSCs when cocultured with Sertoli cells *in vitro*.[77]

Other efforts have been made to induce MSCs to differentiate into male germ cells *in vitro*. BMPs have been shown to be important for stem cell differentiation.[78–80] BMP4 and bone morphogenetic protein 8b (BMP8b) promote the expression of PGCs-specific genes in bone marrow MSCs (BM-MSCs),[79] and transforming growth factor beta1 (TGFβ1) induces the generation of the SSCs and spermatogonia from BM-MSCs.[80] Moreover, canine adipose MSCs can be differentiated into male germ-like cells by BMP4 treatment.[81] BMP4 increases *Stra8* gene expression significantly in mouse BM-MSCs when cocultured with testicular cells.[82] *DAZL* and *BOULE* are members of the *DAZ* gene family, and they play a key role in mediating normal spermatogenesis. Overexpression of BOULE, DAZL, and STRA8 promotes the differentiation of goat BM-MSCs to germ cell-like cells.[83,84] Notably, we have recently demonstrated that human Sertoli cells can be reprogrammed via overexpression of *DAZ* family genes to become SSCs with the self-renewal and differentiation potentials into spermatocytes and spermatids *in vitro*.[85]

SUMMARY AND PERSPECTIVES

The approaches, including the overexpression of male germ cell-related genes, the addition of growth factors and cytokines, coculture with testicular somatic cells, the two-dimensional (2D)- or three-dimensional (3D)-culture system, are useful and efficient for the differentiation of several kinds of stem cells into male germ cells *in vitro* (**Figure 1** and **2**), which might offer male gametes for the treatment of male infertility. Functional spermatids can be induced from human SSCs and ESCs, but the efficiency and safety need to be further improved. For the iPSCs and MSCs, they can be induced to produce male germ cells. Nevertheless, the function of male germ cells remains unknown.

With the development of next-generation sequencing, more information will be available about the regulation of the SSC fate determinations and the process of spermatogenesis.[86,87] It is feasible to establish human SSC lines that can be differentiated *in vitro* and to establish culture systems which coax the efficient differentiation of stem cells into functional spermatids *in vitro*. Overexpression or knockout of the key genes in the MSCs or the iPSCs may reprogram them to develop male gametes with fertility and developmental capacities. These stem cells-derived male gametes might have significant application in reproductive medicine.

AUTHOR CONTRIBUTIONS

YHC and WC wrote the manuscript. SW and CLW helped the drawing of figures. ZH wrote and revised the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

This work was supported by the grants from the National Nature Science Foundation of China (32170862 and 31872845), Major Scientific and Technological Projects for Collaborative Prevention and Control of Birth Defect in Hunan Province (2019SK1012), Key Grant of Research and Development in Hunan Province (2020DK2002), High-Level Talent Gathering Project in Hunan Province (2018RS3066), and Natural Science Foundation of Hunan Province of China (2020JJ5380 and 2020JJ5383).

REFERENCES

1 Vander Borght M, Wyns C. Fertility and infertility: definition and epidemiology. *Clin Biochem* 2018; 62: 2–10.
2 Sun H, Gong TT, Jiang YT, Zhang S, Zhao YH, *et al*. Global, regional, and national prevalence and disability-adjusted life-years for infertility in 195 countries and territories, 1990-2017: results from a global burden of disease study, 2017. *Aging* 2019; 11: 10952–91.
3 Wosnitzer M, Goldstein M, Hardy MP. Review of azoospermia. *Spermatogenesis* 2014; 4: e28218.
4 Thomson AB, Campbell AJ, Irvine DC, Anderson RA, Kelnar CJ, *et al*. Semen quality and spermatozoal DNA integrity in survivors of childhood cancer: a case-control study. *Lancet* 2002; 360: 361–7.
5 Toyooka Y, Tsunekawa N, Akasu R, Noce T. Embryonic stem cells can form germ cells *in vitro*. *Proc Natl Acad Sci U S A* 2003; 100: 11457–62.

6 Geijsen N, Horoschak M, Kim K, Gribnau J, Eggan K, *et al.* Derivation of embryonic germ cells and male gametes from embryonic stem cells. *Nature* 2004; 427: 148–54.
7 Leitch HG, Tang WW, Surani MA. Primordial germ-cell development and epigenetic reprogramming in mammals. *Curr Top Dev Biol* 2013; 104: 149–87.
8 Saitou M, Yamaji M. Primordial germ cells in mice. *Cold Spring Harb Perspect Biol* 2012; 4: a008375.
9 Western PS, Miles DC, van den Bergen JA, Burton M, Sinclair AH. Dynamic regulation of mitotic arrest in fetal male germ cells. *Stem cells* 2008; 26: 339–47.
10 Yoshida S. Stem cells in mammalian spermatogenesis. *Dev Growth Differ* 2010; 52: 311–7.
11 Matsui Y, Zsebo K, Hogan BL. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. *Cell* 1992; 70: 841–7.
12 Resnick JL, Bixler LS, Cheng L, Donovan PJ. Long-term proliferation of mouse primordial germ cells in culture. *Nature* 1992; 359: 550–1.
13 Kehler J, Tolkunova E, Koschorz B, Pesce M, Gentile L, *et al.* Oct4 is required for primordial germ cell survival. *EMBO Rep* 2004; 5: 1078–83.
14 Koshimizu U, Watanabe M, Nakatsuji N. Retinoic acid is a potent growth activator of mouse primordial germ cells *in vitro*. *Dev Biol* 1995; 168: 683–5.
15 Fujiwara T, Dunn NR, Hogan BL. Bone morphogenetic protein 4 in the extraembryonic mesoderm is required for allantois development and the localization and survival of primordial germ cells in the mouse. *Proc Natl Acad Sci U S A* 2001; 98: 13739–44.
16 Tilgner K, Atkinson SP, Golebiewska A, Stojkovic M, Lako M, *et al.* Isolation of primordial germ cells from differentiating human embryonic stem cells. *Stem Cells* 2008; 26: 3075–85.
17 Irie N, Weinberger L, Tang WW, Kobayashi T, Viukov S *et al.* SOX17 is a critical specifier of human primordial germ cell fate. *Cell* 2015; 160: 253–68.
18 Matoba S, Ogura A. Generation of functional oocytes and spermatids from fetal primordial germ cells after ectopic transplantation in adult mice. *Biol Reprod* 2011; 84: 631–8.
19 Nakatsuji N, Chuma S. Differentiation of mouse primordial germ cells into female or male germ cells. *Int J Dev Biol* 2001; 45: 541–8.
20 Yuan Y, Li L, Cheng Q, Diao F, Zeng Q, *et al. In vitro* testicular organogenesis from human fetal gonads produces fertilization-competent spermatids. *Cell Res* 2020; 30: 244–55.
21 Kubota H, Avarbock MR, Brinster RL. Growth factors essential for self-renewal and expansion of mouse spermatogonial stem cells. *Proc Natl Acad Sci U S A* 2004; 101: 16489–94.
22 Sadri-Ardekani H, Mizrak SC, van Daalen SK, Korver CM, Roepers-Gajadien HL, *et al.* Propagation of human spermatogonial stem cells *in vitro*. *JAMA* 2009; 302: 2127–34.
23 Fu H, Zhou F, Yuan Q, Zhang W, Qiu Q, *et al.* MiRNA-31-5p mediates the proliferation and apoptosis of human spermatogonial stem cells via targeting JAZF1 and Cyclin A2. *Mol Ther Nucleic Acids* 2019; 14: 90–100.
24 Chen W, Cui Y, Liu B, Li C, Du L, *et al.* Hsa-miR-1908-3p mediates the self-renewal and apoptosis of human spermatogonialstem cells via targeting KLF2. *Mol Ther Nucleic Acids* 2020; 20: 788–800.
25 Zhou F, Chen W, Cui Y, Liu B, Yuan Q, *et al.* MiRNA-122-5p stimulates the proliferation and DNA synthesis and inhibits the early apoptosis of human spermatogonial stem cells by targeting CBL and competing with lncRNA CASC7. *Aging* 2020; 12: 25528–46.
26 Zhou F, Yuan Q, Zhang W, Niu M, Fu H, *et al.* MiR-663a stimulates proliferation and suppresses early apoptosis of human spermatogonialstem cells by targeting NFIX and regulating cell cycle. *Mol Ther Nucleic Acids* 2018; 12: 319–36.
27 Fu H, Zhang W, Yuan Q, Niu M, Zhou F, *et al.* PAK1 promotes the proliferation and inhibits apoptosis of human spermatogonialstem cells via PDK1/KDR/ZNF367 and ERK1/2 and AKT pathways. *Mol Ther Nucleic Acids* 2018; 12: 769–86.
28 Tian R, Yao C, Yang C, Zhu Z, Li C, *et al.* Fibroblast growth factor-5 promotes spermatogonial stem cell proliferation via ERK and AKT activation. *Stem Cell Res Ther* 2019; 10: 40.
29 Feng LX, Chen Y, Dettin L, Pera RA, Herr JC, *et al.* Generation and *in vitro* differentiation of a spermatogonial cell line. *Science* 2002; 297: 392–5.
30 Wang P, Suo LJ, Shang H, Li Y, Li GX, *et al.* Differentiation of spermatogonial stem cell-like cells from murine testicular tissue into haploid male germ cells *in vitro*. *Cytotechnology* 2014; 66: 365–72.
31 Ibtisham F, Zhao Y, Nawab A, Wu J, Mei X, *et al. In vitro* production of haploid germ cells from murine spermatogonial stem cells using a two-dimensional cell culture system. *Theriogenology* 2021; 162: 84–94.
32 Sato T, Katagiri K, Yokonishi T, Kubota Y, Inoue K, *et al. In vitro* production of fertile sperm from murine spermatogonial stem cell lines. *Nat Commun* 2011; 2: 472.
33 Sun M, Yuan Q, Niu M, Wang H, Wen L, *et al.* Efficient generation of functional haploid spermatids from human germline stem cells by three-dimensional-induced system. *Cell Death Differ* 2018; 25: 749–66.
34 Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981; 292: 154–6.
35 Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci U S A* 1981; 78: 7634–8.
36 Clark AT, Bodnar MS, Fox M, Rodriquez RT, Abeyta MJ, *et al.* Spontaneous differentiation of germ cells from human embryonic stem cells *in vitro*. *Hum Mol Genet* 2004; 13: 727–39.
37 Mizuno K, Tokumasu A, Nakamura A, Hayashi Y, Kojima Y, *et al.* Genes associated with the formation of germ cells from embryonic stem cells in cultures containing different glucose concentrations. *Mol Reprod Dev* 2006; 73: 437–45.
38 Nayernia K, Nolte J, Michelmann HW, Lee JH, Rathsack K, *et al. In vitro*-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. *Dev Cell* 2006; 11: 125–32.
39 Yamauchi K, Hasegawa K, Chuma S, Nakatsuji N, Suemori H. *In vitro* germ cell differentiation from cynomolgus monkey embryonic stem cells. *PLoS One* 2009; 4: e5338.
40 Yu Z, Ji P, Cao J, Zhu S, Li Y, *et al.* Dazl promotes germ cell differentiation from embryonic stem cells. *J Mol Cell Biol* 2009; 1: 93–103.
41 Zhou Q, Wang M, Yuan Y, Wang X, Fu R, *et al.* Complete meiosis from embryonic stem cell-derived germ cells *in vitro*. *Cell Stem Cell* 2016; 18: 330–40.
42 Li N, Ma W, Shen Q, Zhang M, Du Z, *et al.* Reconstitution of male germline cell specification from mouse embryonic stem cells using defined factors *in vitro*. *Cell Death Differ* 2019; 26: 2115–24.
43 Aflatoonian B, Ruban L, Jones M, Aflatoonian R, Fazeli A, *et al. In vitro* post-meiotic germ cell development from human embryonic stem cells. *Hum Reprod* 2009; 24: 3150–9.
44 Richards M, Fong CY, Bongso A. Comparative evaluation of different *in vitro* systems that stimulate germ cell differentiation in human embryonic stem cells. *Fertil Steril* 2010; 93: 986–94.
45 Duggal G, Heindryckx B, Warrier S, Taelman J, Van der Jeught M, *et al.* Exogenous supplementation of Activin A enhances germ cell differentiation of human embryonic stem cells. *Mol Hum Reprod* 2015; 21: 410–23.
46 West FD, Roche-Rios MI, Abraham S, Rao RR, Natrajan MS, *et al.* KIT ligand and bone morphogenetic protein signaling enhances human embryonic stem cell to germ-like cell differentiation. *Hum Reprod* 2010; 25: 168–78.
47 Zhang S, Yu M, Liu C, Wang L, Hu Y, *et al.* MIR-34c regulates mouse embryonic stem cells differentiation into male germ-like cells through RARg. *Cell Biochem Funct* 2012; 30: 623–32.
48 Chen H, Zuo Q, Wang Y, Ahmed MF, Jin K, *et al.* Regulation of hedgehog signaling in chicken embryonic stem cells differentiation into male germ cells (*Gallus*). *J Cell Biochem* 2017; 118: 1379–86.
49 Zhang Y, Zhang L, Zuo Q, Wang Y, Zhang Y, *et al.* JAK-STAT signaling regulation of chicken embryonic stem cell differentiation into male germ cells. *In Vitro Cell Dev Biol Anim* 2017; 53: 728–43.
50 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; 126: 663–76.
51 Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; 318: 1917–20.
52 Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; 131: 861–72.
53 Imamura M, Aoi T, Tokumasu A, Mise N, Abe K, *et al.* Induction of primordial germ cells from mouse induced pluripotent stem cells derived from adult hepatocytes. *Mol Reprod Dev* 2010; 77: 802–11.
54 Zhu Y, Hu HL, Li P, Yang S, Zhang W, *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: 574–9.
55 Yang S, Bo J, Hu H, Guo X, Tian R, *et al.* Derivation of male germ cells from induced pluripotent stem cells *in vitro* and in reconstituted seminiferous tubules. *Cell Prolif* 2012; 45: 91–100.
56 Cai H, Xia X, Wang L, Liu Y, He Z, *et al. In vitro* and *in vivo* differentiation of induced pluripotent stem cells into male germ cells. *Biochem Biophys Res Commun* 2013; 433: 286–91.
57 Niu Z, Hu Y, Chu Z, Yu M, Bai Y, *et al.* Germ-like cell differentiation from induced pluripotent stem cells (iPSCs). *Cell Biochem Funct* 2013; 31: 12–9.
58 Li P, Hu H, Yang S, Tian R, Zhang Z, *et al.* Differentiation of induced pluripotent stem cells into male germ cells *in vitro* through embryoid body formation and retinoic acid or testosterone induction. *Biomed Res Int* 2013; 2013: 608728.
59 Li Y, Wang X, Feng X, Liao S, Zhang D, *et al.* Generation of male germ cells from mouse induced pluripotent stem cells *in vitro*. *Stem Cell Res* 2014; 12: 517–30.
60 Mahabadi JA, Karimian M, Aghighi F, Enderami SE, Seyyed Hosseini E, *et al.* Retinoic acid and 17beta-estradiol improve male germ cell differentiation from mouse-induced pluripotent stem cells. *Andrologia* 2020; 52: e13466.
61 Mahabadi JA, Tameh AA, Talaei SA, Karimian M, Rahiminia T, *et al.* Retinoic acid and/or progesterone differentiate mouse induced pluripotent stem cells into male germ cells *in vitro*. *J Cell Biochem* 2020; 121: 2159–69.
62 Wang H, Xiang J, Zhang W, Li J, Wei Q, *et al.* Induction of germ cell-like cells from porcine induced pluripotent stem cells. *Sci Rep* 2016; 6: 27256.
63 Eguizabal C, Montserrat N, Vassena R, Barragan M, Garreta E, *et al.* Complete meiosis from human induced pluripotent stem cells. *Stem Cells* 2011; 29: 1186-95.
64 Ganjibakhsh M, Mehraein F, Koruji M, Aflatoonian R, Farzaneh P. Three-dimensional decellularized amnion membrane scaffold promotes the efficiency of male

germ cells generation from human induced pluripotent stem cells. *Exp Cell Res* 2019; 384: 111544.
65 Huang P, Lin LM, Wu XY, Tang QL, Feng 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: 747–54.
66 Liu H, Chen M, Liu L, Ren S, Cheng P, *et al*. Induction of human adipose-derived mesenchymal stem cells into germ lineage using retinoic acid. *Cell Reprogram* 2018; 20: 127–34.
67 Dyce PW, Tenn N, Kidder GM. Retinoic acid enhances germ cell differentiation of mouse skin-derived stem cells. *J Ovarian Res* 2018; 11: 19.
68 Ghatreh KS, Eliyasi MD, Alaei S, Saki G. Differentiation potential of adipose tissue-derived mesenchymal stem cells into germ cells with and without growth factors. *Andrologia* 2021; 53: e13892.
69 Cortez J, Bahamonde J, De Los Reyes M, Palomino J, Torres CG, *et al*. *In vitro* differentiation of bovine bone marrow-derived mesenchymal stem cells into male germ cells by exposure to exogenous bioactive factors. *Reprod Domest Anim* 2018; 53: 700–9.
70 Xie L, Lin L, Tang Q, Li W, Huang T, *et al*. Sertoli cell-mediated differentiation of male germ cell-like cells from human umbilical cord Wharton's jelly-derived mesenchymal stem cells in an *in vitro* co-culture system. *Eur J Med Res* 2015; 20: 9.
71 Segunda MN, Bahamonde J, Munoz I, Sepulveda S, Cortez J *et al*. Sertoli cell-mediated differentiation of bovine fetal mesenchymal stem cells into germ cell lineage using an *in vitro* co-culture system. *Theriogenology* 2019; 130: 8–18.
72 Majidi F, Bamehr H, Shalchian Z, Kouchakian MR, Mohammadzadeh N, *et al*. Differentiation of human umbilical cord mesenchymal stem cell into germ-like cell under effect of co-culture with testicular cell tissue. *Anat Histol Embryol* 2020; 49: 359–64.
73 Maghami RG, Mirzapour T, Bayrami A. Differentiation of mesenchymal stem cells to germ-like cells under induction of Sertoli cell-conditioned medium and retinoic acid. *Andrologia* 2018; 50: e12887.
74 Dissanayake D, Patel H, Wijesinghe PS. Differentiation of human male germ cells from Wharton's jelly-derived mesenchymal stem cells. *Clin Exp Reprod Med* 2018; 45: 75–81.
75 Salem M, Mirzapour T, Bayrami A, Sagha M. Germ cell differentiation of bone marrow mesenchymal stem cells. *Andrologia* 2019; 51: e13229.
76 Ghorbanlou M, Abdanipour A, Shirazi R, Malekmohammadi N, Shokri S, *et al*. Indirect co-culture of testicular cells with bone marrow mesenchymal stem cells leads to male germ cell-specific gene expressions. *Cell J* 2019; 20: 505–12.
77 Luo Y, Xie L, Mohsin A, Ahmed W, Xu C, *et al*. Efficient generation of male germ-like cells derived during co-culturing of adipose-derived mesenchymal stem cells with Sertoli cells under retinoic acid and testosterone induction. *Stem Cell Res Ther* 2019; 10: 91.
78 Afsartala Z, Rezvanfar MA, Hodjat M, Tanha S, Assadollahi V, *et al*. Amniotic membrane mesenchymal stem cells can differentiate into germ cells *in vitro*. *In Vitro Cell Dev Biol Anim* 2016; 52: 1060–71.
79 Mazaheri Z, Movahedin M, Rahbarizadeh F, Amanpour S. 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: 521–5.
80 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: 588–98.
81 Wei Y, Fang J, Cai S, Lv C, Zhang S, *et al*. Primordial germ cell-like cells derived from canine adipose mesenchymal stem cells. *Cell Prolif* 2016; 49: 503–11.
82 Malekmohamadi N, Abdanipour A, Ghorbanlou M, Shokri S, Shirazi R, *et al*. Differentiation of bone marrow derived mesenchymal stem cells into male germ-like cells in co-culture with testicular cells. *Endocr Regul* 2019; 53: 93–9.
83 Li PZ, Yan GY, Han L, Pang J, Zhong BS, *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: 300–12.
84 Zhang YL, Li PZ, Pang J, Wan YJ, Zhang GM, *et al*. Induction of goat bone marrow mesenchymal stem cells into putative male germ cells using mRNA for STRA8, BOULE and DAZL. *Cytotechnology* 2019; 71: 563–72.
85 Zhang W, Chen W, Cui Y, Wen L, Yuan Q, *et al*. Direct reprogramming of human Sertoli cells into male germline stem cells with the self-renewal and differentiation potentials via overexpressing DAZL/DAZ2/BOULE genes. *Stem Cell Rep* 2021; 16: 2798–812.
86 Wen L, Tang F. Human germline cell development: from the perspective of single-cell sequencing. *Mol Cell* 2019; 76: 320–8.
87 Du L, Chen W, Cheng Z, Wu S, He J, *et al*. Novel gene regulation in normal and abnormal spermatogenesis. *Cells* 2021; 10: 666.