Advances in Inducers for Differentiation of Induced Pluripotent Stem Cells into Male Germ Cells: Postprint

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Abstract

Induced pluripotent stem cells (iPSCs) are artificially obtained through cellular reprogramming technology and functionally resemble embryonic stem cells (ESCs). They can differentiate into all cell types, including those of the three germ layers, circumvent the ethical controversies and immune rejection issues associated with ESCs, and thus hold tremendous promise for broad applications. This review summarizes the inducers used for in vitro differentiation of iPSCs into germ cells and their induction efficacies. Research on the mechanisms of germ cell development is expected to advance future reproductive and developmental technologies.

Full Text

Preamble

Progress in Inducers Stimulating Differentiation of iPS Cells into Male Germ Cells

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Abstract: Induced pluripotent stem cells (iPSCs) are artificially generated through cellular reprogramming and share similar functions with embryonic stem cells (ESCs). They can differentiate into all cell types including those from all three germ layers, while circumventing the ethical controversies associated with ESCs and immune rejection following transplantation, thus offering

tremendous application potential. This review summarizes recent advances in the in vitro differentiation of iPSCs into male germ cells, focusing on various inducers and their differentiation efficiency. Investigating the mechanisms underlying germ cell development holds promise for advancing future reproductive and developmental technologies.

Keywords: iPSCs; male germ cells; differentiation; inducers

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1. iPSCs

1.1 Advantages and Limitations of iPSCs

In 2006, a research team from Kyoto University in Japan first reprogrammed somatic cells into pluripotent stem cells, which they defined as induced pluripotent stem cells [1]. iPSCs possess self-renewal capacity and can undergo symmetric division to produce daughter cells identical to the parent cell, giving them essentially "unlimited" proliferative potential and "immortality." These cells also exhibit multi-lineage differentiation potential, capable of differentiating under specific induction conditions into mature cells of various phenotypes, such as neuroepithelial cells [2], pancreatic cells [3], hepatocytes [4], cardiomyocytes [5], vascular endothelial cells [6], and smooth muscle cells from different germ layers [7]. iPSCs function almost identically to ESCs, expressing various ESC surface markers and differentiating into various tissue cells. Their use is not constrained by immune rejection or ethical limitations, providing unique advantages in tissue engineering and regenerative medicine.

iPSCs have profoundly influenced clinical medicine development [12]. Since ESCs can differentiate into cells of all three germ layers, they can serve as a therapeutic approach for animal models; however, clinical applications require resolution of ethical issues and post-transplantation immune rejection. iPSCs can be derived from the patient' s own cells, effectively circumventing ethical concerns and minimizing immune rejection, which greatly benefits pharmaceutical and regenerative medicine research. However, iPSCs have three notable defects: first, the reprogramming efficiency of human cells is very low, making it difficult to generate patient-specific iPSCs from primary cells and initial cell populations; second, the integration of exogenous genes into the somatic cell genome, particularly oncogenes such as c-Myc and KLF4, may induce tumor formation [13]; and third, even if iPSCs form teratomas, a small number of undifferentiated cells can cause tumors, making it crucial to direct iPSCs toward beneficial cell types rather than allowing large numbers of undifferentiated cells

to persist. Although some successful iPSC models exist in chimeric mice, the tumorigenic risk cannot be completely eliminated [14].

1.2 iPSCs for Germ Cell Differentiation

Besides the ectopic expression of Oct-4, Sox2, KLF4, and c-Myc, other transcription factor mutations can also generate iPSCs, such as selection of primary somatic cells, reprogramming factors, and transduction methods [15]. Researchers believe that new iPSCs will eventually replace ESCs as the primary source material for inducing germ cells. Before clinical application of iPSC-derived germ cells, scientific evaluation must be completed based on their meiotic mechanisms, post-reprogramming processes, and nuclear and mitochondrial functions. The expression of marker genes or immunostaining of marker proteins such as C-kit, DDX4, and SSEA1 can be used to identify PGC formation [16]. Characteristics of cells entering the meiotic phase include detection of haploid differentiated cells and meiosis-related markers, including transition protein 1 (TP1), protamine 1 (Prot1), and acrosin [17].

Since mouse iPSCs can differentiate into germ cells through tetraploid complementation [18], iPSCs have become a feasible option for studying germ cell differentiation mechanisms in vitro. Kim et al. obtained iPSCs derived from neural stem cells, which showed elevated expression of Sycp3, GDF9, and early PGC marker genes during differentiation toward germ cells [19]. Imamura [11] et al. successfully induced mouse iPSCs from adult hepatocytes under the regulation of VASA and OCT4 gene promoters [15]. Studies found that VASA-regulated PGCs could produce transgenic iPSCs in embryoid bodies (EBs) or under feeder layer conditions without leukemia inhibitory factor (LIF). A small number of OCT4/VASA oocyte-like cells appeared at the edges of EBs. To promote germ cell differentiation, researchers attempted to stimulate iPSCs with epidermal growth factor (EGF), bone morphogenetic protein 4 (BMP4), and glial cell-derived neurotrophic factor (GDNF) [16]. Interestingly, VASA-labeled cells appeared in cell colonies. After 21 days, numerous cell colonies were observed, with expression of meiotic marker genes such as Sycp1 and Dmc1 detected, and migrating PGCs could be observed, though post-meiotic markers (TP1, Protamine1, etc.) were not detected.

2. Inducers for iPSC Differentiation into Male Germ Cells

2.1 Retinoic Acid (RA)

Retinoic acid (RA) is a non-peptide lipophilic small molecule extracted from vitamin A (retinol) that serves as a ligand for nuclear retinoic acid receptors (RARs) [17], functioning by binding to ligand-dependent transcription factors RAR , RAR , and RAR [18]. Different spermatogenic epithelial cells express different RARs: Sertoli cells express RAR , round spermatids express RAR , and type A spermatogonia express RAR [19]. RA reportedly acts as a testicular stimulant immediately after birth to stimulate downstream gene expression.

Additionally, RA can stimulate meiosis and STRA8 gene expression. Koubova et al. confirmed that RA-activated STRA8 in embryoid bodies ensures proper timing of sexual development. Numerous studies have verified RA' s mode of action in stimulating meiosis initiation [20] and its influence on PGC proliferation and development [21]. Tan and Bowles et al. demonstrated that RA can induce iPSCs or ESCs to differentiate into PGCs [17]. During mouse PGC culture, RA promotes PGC proliferation and slows PGC degeneration, acting as a cytokine both in vivo and in vitro. Moreover, RA can prevent apoptosis in mouse PGCs [22]. Studies show that RA both promotes differentiation of stem cells into PGCs and PGC proliferation [23], and can act on EB cells to promote ESC differentiation [24].

Although iPSCs possess the capacity to differentiate into numerous male germ cells, whether this differentiation occurs spontaneously or results from RA induction requires further investigation. Li et al. showed that following RA or testosterone treatment, the induction results of mouse iPSCs differentiating into male germ cells through EB formation could even identify these cells as likely haploid [25]. One drawback of RA is its role in neuronal differentiation, which may lead to iPSC differentiation into neural cells. RA is recognized as the most potent morphogen for inducing neural progenitors and neural cells from stem cells.

2.2 Bone Morphogenetic Proteins (BMPs)

BMPs are multifunctional cytokines belonging to the transforming growth factor- (TGF-) superfamily, released by early embryonic ectoderm and playing important roles in germ cell development and function [26]. When animals are implanted with aged bone, a cellular response induces new bone formation due to the action of one or a class of proteins called bone morphogenetic proteins [27]. In vitro treatment with BMPs can induce iPSCs to differentiate into male germ cells [28]. In mammals, the induction of specific epithelial cells into PGCs is regulated by BMPs, including BMP8b and BMP4, both expressed through extraembryonic ectoderm (ExE) [29]. Male germ cells express certain proteins of the Gbb-60A subfamily, which are closely related to BMP8a and BMP8b involved in spermatogenesis and maintaining germ cell proliferation and survival [30]. Cytokines such as BMP4 can make human and mouse ESCs more susceptible to germ cell differentiation. Therefore, BMPs are important factors in cell proliferation, apoptosis, differentiation, and morphogenesis.

In mouse epiblast transplantation culture, BMP4 and BMP8b proteins can induce PGC formation [31]. During the initial 3 days of differentiation, the germ cell marker gene VASA is expressed by human recombinant BMP4 protein in a dose-dependent manner, and combined use of BMP7, BMP8b, and BMP4 can further enhance VASA expression [32].

To confirm whether BMP addition could induce iPSC differentiation into germ cells, Panula et al. [33] used iPSCs (IMR90) and iHUF4 cells differentiated for 7

and 14 days, divided into groups with and without BMP4, BMP7, and BMP8b, to observe the effects of BMP supplementation on germ cell induction from iPSCs and hESCs. The experimental group detected low expression of DAZL and VASA genes in undifferentiated iHUF4 cells, and low VASA expression in undifferentiated iPSCs (IMR90). Extended culture, BMP addition (BMP4, BMP7, BMP8b), and co-culture with human or mouse fetal gonadal stromal cells could promote spontaneous PGC differentiation [34]. Western blotting after BMP induction showed elevated expression of the germ cell marker gene VASA, providing evidence for the specific function of BMPs in germ cells.

One disadvantage of BMPs is their high cost, and their mechanism of action and side effects are not yet fully understood.

2.3 Human Fetal Gonadal Cells (hFGCs)

Research has confirmed that iPSCs derived from fetal somatic cells can differentiate into early-stage PGCs, indicating that iPSCs have similar clinical therapeutic value to hESCs [35]. However, differentiation of iPSCs obtained from reprogrammed human cells into germ cells has not been reported [31]. Park et al. showed that compared with two induction methods—specific culture and adherent culture—the induction efficiency of iPGCs using iPSCs and ESCs was higher. Although human fetal gonadal cells (hFGCs) can produce large numbers of iPGCs, iPGCs can also be obtained after induction from non-gonadal stromal cells of fetal liver and placental tissue.

hFGCs differentiated from iPGC-positive cells are comparable to germ cells from the first trimester [36]. Co-culture of hiPSCs with hESCs under appropriate differentiation conditions and labeling with hFGCs can significantly improve iPGC differentiation capacity. After 7 days of hFGC differentiation, iPGCs develop into immature PGCs, a process equivalent to development from in vitro fertilization to 9 weeks of gestation [34]. Hikabe et al. successfully reconstructed the female germline in vitro, but the applications of this culture system remain limited.

2.4 Testosterone

Testosterone is a male sex hormone essential for the development of the male reproductive system and formation of secondary sexual characteristics [37]. Androgen receptor expression is present in both blastocyst inner cell mass and ES cell lines [38]. In Leydig cells of the testis, testosterone under the combined influence of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) can promote Sertoli cell-induced gene transcription and growth factor secretion, thereby promoting germ cell differentiation. Sani et al. showed that the synergistic action of testosterone and FSH in adult rats can prevent germ cell degeneration and/or stimulate germ cell development [39]. High concentrations of testosterone, FSH, and co-culture with Sertoli cells all promote germ cell survival and accelerate meiotic progression [40].

In vitro, the combination of testosterone and Sertoli cells, along with direct stimulation of germ cells, can promote germ cell differentiation to the haploid stage. Studies have shown that co-culture with Sertoli cells and hormones can induce completion of meiosis in in vitro matured sperm and oocytes [41]. Sliva et al. found that testosterone and RA can affect the expression capacity of male germ cell marker genes in ESC-differentiated cells. Cell proliferation and differentiation are regulated by sequential changes in sex steroid hormones [41]. Li et al. reported that iPSCs can differentiate into male germ cells through EB formation and induction with RA or testosterone. Studies show that after RA or testosterone induction, approximately 2-8% of EB cells are haploid [25]. Research confirms that testosterone can enhance the ability of iPSCs to differentiate into male germ cells and may be used in future research on male infertility.

2.5 Stem Cell Factor (SCF)

Stem cell factor (SCF) is a membrane-bound cytokine on Sertoli cells whose tyrosine kinase receptor is c-kit, adjacent to the germ cell surface. SCF strongly regulates germ cell proliferation and apoptosis [43]. SCF regulates expression of the stem and germ cell marker gene c-kit and can improve cell survival and proliferation [44]. Mature PGCs in mice are defined as alkaline phosphatase-positive cell populations at E7.25, which can be marked by re-expression of the Stella gene, a maternal effect gene actively expressed from the zygote stage until the blastocyst stage [45]. SCF can promote PGC proliferation and improve survival rates.

Studies show that mouse basic fibroblast growth factor and embryonic fibroblast feeder layers are essential for PGC culture, but their role in hESC differentiation into germ cells remains unclear [46]. Research demonstrates that adding bFGF on MEF feeder layers with continuous culture can increase the proportion of POU5F1-positive and DDX4-positive cells differentiated from hESCs to 69% [47]. Under feeder-free culture conditions, bFGF supplementation is more important for isolation and maintenance of POU5F1-positive and DDX4-positive cells [10]. Additionally, combined application of SCF, BMP4, and BMP8b has proven extremely effective during iPGC-LC induction [16].

2.6 Glial Cell-Derived Neurotrophic Factor (GDNF)

GDNF is a member of the transforming growth factor- family that, together with three related molecules, forms the GDNF family ligand subfamily (GFLs). Adding GDNF to culture medium supplemented with EGF or bFGF can promote in vitro proliferation of mouse spermatogonial stem cells (SSCs) and affect the function of undifferentiated spermatogonia in vivo. Therefore, GDNF is commonly used as a supplement in mouse SSC culture medium along with FGF, EGF, and LIF [48]. Wang et al. [49] showed that GDNF is required for SSC proliferation and maintenance during the induction of porcine iPSCs into germ cells. Boozarpour et al. [50] first demonstrated that GDNF could distin-

guish mesenchymal stem cells from chicken SSCs, and that GDNF had stronger ability than RA to induce SSCs from chicken mesenchymal stem cells, though this inference requires further verification.

2.7 Potential Mechanisms of iPSC Differentiation into Male Germ Cells

As a new member of the stem cell family, iPSCs are very similar to ESCs in terms of colony morphology, gene expression patterns, surface markers, embryoid body formation, teratoma and chimera formation (in mice), and differentiation capacity [51]. ESC induction into germ cells involves ethical controversies and post-transplantation immune rejection issues, while adult stem cells, though widely available and easily accessible, have limited pluripotency [52-53]. iPSCs combine the advantages of both ESCs and adult stem cells, avoiding ethical controversies and immune rejection while being easy to obtain. Although their induction efficiency is low and requires introduction of numerous exogenous genes that may affect subsequent germ cell differentiation, iPSCs provide an excellent research platform for revealing male germ cell development mechanisms and studying male infertility, representing a major step forward in clinical stem cell applications. Animal iPSC-SSC-spermatid research also has tremendous application value in animal transgenic studies [54].

Current research indicates that the retinol metabolism pathway [55], BMP4 metabolism pathway [56], and TGF pathway play important roles in iPSC differentiation into male germ cells. Studies show that RA, the end product of the retinol metabolism pathway, can induce iPSC differentiation toward male germ cells in vitro and generate functional SSC-like cells. In the retinoic acid metabolism process of avian male germ cell differentiation, members of the cytochrome P450 family, ADH, and ALDH all participate in regulation to some degree. RA can regulate the timing of meiosis initiation. For germ cells in embryonic ovaries, decreased Cyp26b1 expression stimulates RA signaling, leading to Stra8 gene expression and initiation of meiosis in embryonic ovarian germ cells. For germ cells in embryonic testes, metabolic enzyme Cyp26b1 expressed in testicular somatic cells degrades RA, suppressing RA signaling and delaying meiosis. At sexual maturity, Cyp26b1 expression decreases and RA signaling is activated [57-58].

In early postnatal stages, Sertoli cells secrete BMP4, which is downregulated before puberty. BMP4 regulates PGC and oogonia differentiation and development by acting through Alk3 and R-Smad receptors [59]; inhibition of BMP4 signaling can cause partial or complete loss of germ cells [60].

[Figure 1: see original paper] Schematic diagram of iPSC differentiation into male germ cells

This review summarizes research progress on iPSC differentiation into male germ cells, aiming to discuss the characteristics of different inducers for iPSC differentiation. hiPSC cells offer good clinical prospects as an in vitro model for

studying infertile patients. Induction technology for hESCs/iPSCs into male germ cells can promote understanding of related diseases and infertility, as well as development of new therapies. Research on male germ cells aims to overcome infertility by differentiating iPSCs into patient-specific male gametes. However, clinical application of iPSCs faces challenges, such as extremely complex induction processes and the need for development and improvement of induction methods. For these reasons, we recommend optimizing culture and differentiation methods, procedures, and targeted selection of exogenous inducers. Whether iPSCs generate male germ cells spontaneously, and whether their differentiation capacity can elucidate gamete formation ability, require further study. We hope that iPSC survival rates and differentiation efficiency can be substantially improved in the future. Whether different inducers can selectively induce apoptosis in undifferentiated human PSCs without affecting their differentiated derivatives represents a future research hotspot.

References

[1] Wu Y, Li O, He C, et al. Generation and characterization of induced pluripotent stem cells from guinea pig fetal fibroblasts. Molecular Medicine Reports, 2017, 15(6): 3690-3698.

[2] Wernig M, Zhao J P, Pruszak J, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the brain and improve symptoms of rats with Parkinson' s disease. Proceedings of the National Academy of Sciences of the United States of America, 2008, 105(15): 5856-5861.

[3] Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell, 2007, 131(5): 861-872.

[4] Imamura M, Aoi T, Tokumasu A, et al. Induction of primordial germ cells from mouse induced pluripotent stem cells and adult hepatocytes. Molecular Reproduction and Development, 2010; 77: 802-811.

[5] Correia C, Serra M, Espinha N, et al. Combining hypoxia and bioreactor hydrodynamics boosts induced pluripotent stem cell differentiation towards cardiomyocytes. Stem Cell Reviews, 2014; 10(6): 786-801.

[6] Schenke-Layland K, Rhodes K E, Angelis E, et al. Reprogrammed mouse fibroblasts differentiate into cells of the hematopoietic and cardiovascular lineages. Stem Cells, 2008, 26(6): 1537-1546.

[7] Shi Y, Inoue H, Wu JC, Yamanaka S. Induced pluripotent stem cell technology: a decade of progress. Nature Reviews Drug Discovery, 2017, 16(2): 115-130.

[8] Hikabe O, Hamazaki N, Nagamatsu G, et al. Reconstitution in vitro of the entire cycle of the mouse female germ line. Nature, 2016, 539(7628): 299–303.

[9] Cai H, Xia X, Wang L, et al. In vitro and in vivo differentiation of induced

pluripotent stem cells into male germ cells. Biochemical and Biophysical Research Communications, 2013, 433(3): 286–291.

[10] Eguizabal C, Montserrat N, Vassena R, et al. Complete meiosis from human induced pluripotent stem cells. Stem Cells, 2011, (29): 1186–1195.

[11] Imamura M, Hikabe O, Lin ZYC, et al. Generation of germ cells in vitro in the era of induced pluripotent stem cells. Molecular Reproduction and Development, 2014, 81(1): 2–19.

[12] Yin X, Li Y, Li J, et al. Generation and periodontal differentiation of human gingival integration-free induced pluripotent stem cells. Biochemical and Biophysical Research Communications, 2016, 473(3): 726–732.

[13] Yamanaka S. A fresh look at iPS cells. Cell, 2009, 137(1): 13–17.

[14] Liu S-P, Fu R-H, Huang Y-C, et al. Induced pluripotent stem (iPS) cell research overview. Cell Transplantation, 2011, 20(1): 15–19.

[15] Li J, Song W, Pan G, Zhou J. Advances in understanding the cell types and approaches used for generating induced pluripotent stem cells. Journal of Hematology & Oncology, 2014, 7(50): 1–18.

[16] Li Y, Wang X, Feng X, et al. Generation of male germ cells from mouse induced pluripotent stem cells in vitro. Stem Cell Research, 2014, 12(2): 517–530.

[17] Tan H, Wang J-J, Cheng S-F, et al. Retinoic acid promotes the proliferation of primordial germ cell-like cells differentiated from mouse skin-derived stem cells in vitro. Theriogenology, 2016, 85(3): 408–418.

[18] Niederreither K, Dollé P. Retinoic acid in development: towards an integrated view. Nature Reviews Genetics, 2008, 9(7): 541–553.

[19] Kent T, Griswold MD. Checking the pulse of vitamin A metabolism and signaling during mammalian spermatogenesis. Journal of Developmental Biology, 2014, 2: 34–49.

[20] Koubova J, Hu Y-C, Bhattacharyya T, et al. Retinoic acid activates two pathways required for meiosis in mice. PLoS Genetics, 2014, 10(8): e1004541.

[21] Costa JJ, Souza GB, Soares MA, et al. In vitro differentiation of primordial germ cells and oocyte-like cells from stem cells. Histology and Histopathology, 2018, 33(2): 121-132.

[22] Morita Y, Tilly JL. Segregation of retinoic acid effects on fetal ovarian germ cell mitosis versus apoptosis by requirement for new macromolecular synthesis. Endocrinology, 1999, 140(6): 2696–2703.

[23] Zhang Y, Wang Y, Zuo Q, et al. Selection of the inducer for the differentiation of chicken embryonic stem cells into male germ cells in vitro. PLoS ONE, 2016, 11(10): e0164664.

[24] Geijsen N, Horoschak M, Kim K, et al. Derivation of embryonic germ cells and male gametes from embryonic stem cells. Nature, 2004, 427(6970): 148–154.

[25] Li P, Hu H, Yang S, 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 Research International, 2013, 2013: 608728.

[26] Yang S, Yuan Q, Niu M, Hou J, Zhu Z, Sun M, et al. BMP4 promotes mouse iPS cell differentiation to male germ cells via Smad1/5, Gata4, Id1 and Id2. Reproduction, 2017; 153: 211–220.

[27] Wozney JM. Overview of bone morphogenetic proteins. Spine, 2002; 27: 2–8.

[28] He Z. Derivation of male germ cells from induced pluripotent stem (iPS) cells: a novel and crucial source for generating male gametes. Asian Journal of Andrology, 2012, 14(4): 516–517.

[29] Lochab AK, Extavour CG. Bone Morphogenetic Protein (BMP) signaling in animal reproductive system development and function. Developmental Biology, 2017, 427(2): 258–269.

[30] Ying Y, Liu X-M, Marble A, et al. Requirement of Bmp8b for the generation of primordial germ cells in the mouse. Molecular Endocrinology, 2000, 14(7): 1053–1063.

[31] Ying Y, Qi X, Zhao G-Q. Induction of primordial germ cells from murine epiblasts by synergistic action of BMP4 and BMP8B signaling pathways. Proceedings of the National Academy of Sciences USA, 2001, 98(14): 7858–7862.

[32] Kee K, Gonsalves JM, Clark AT, et al. Bone morphogenetic proteins induce germ cell differentiation from human embryonic stem cells. Stem Cells and Development, 2006, 15(6): 831–837.

[33] Panula S, Medrano JV, Kee K, et al. Human germ cell differentiation from fetal- and adult-derived induced pluripotent stem cells. Human Molecular Genetics, 2011, 20(4): 752–762.

[34] Bucay N, Yebra M, Cirulli V, et al. A novel approach for the derivation of putative primordial germ cells from human embryonic stem cells. Cells, 2009, 27(1): 68–77.

[35] Park TS, Galic Z, Conway AE, et al. Derivation of primordial germ cells from human embryonic and induced pluripotent stem cells is significantly improved by coculture with human fetal gonadal cells. Stem Cells, 2009, 27(4): 783–795.

[36] Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science, 2007, 318(5858): 1917–1920.

[37] Hashimoto H, Yuasa S. Testosterone induces cardiomyocyte differentiation from embryonic stem cells. Journal of Molecular and Cellular Cardiology, 2013,

62: 69-71.

[38] Silva C, Wood JR, Salvador L, et al. Expression profile of male germ cell-associated genes in mouse embryonic stem cell cultures treated with all-trans retinoic acid and testosterone. Molecular Reproduction and Development, 2009, 76(1): 11-21.

[39] Narenji Sani R, Tajik P, Movahedin M, et al. Effect of follicle stimulating hormone and testosterone on viability rate of cryopreserved spermatogonial stem cell after thawing. Iranian Journal of Veterinary Science and Technology, 2013, 5: 26-34.

[40] Tajik P, Sani RN, Moezifar M, et al. Effect of follicle-stimulating hormone and testosterone on colony formation of bovine spermatogonial stem cell. Comparative Clinical Pathology, 2014; 23: 901-906.

[41] Zanganeh BM, Rastegar T, Roudkenar MH, et al. Co-culture of spermatogonial stem cells with sertoli cells in the presence of testosterone and FSH improved differentiation via up-regulation of post meiotic genes. Acta Medica Iranica, 2013, 51(1): 1-11.

[42] Gargett CE, Chan RW, Schwab KE. Hormone and growth factor signaling in endometrial renewal: role of stem/progenitor cells. Molecular and Cellular Endocrinology, 2008, 288(1-2): 22-29.

[43] Correia S, Alves MR, Cavaco JE, et al. Estrogenic regulation of testicular expression of stem cell factor and c-kit: implications in germ cell survival and male fertility. Fertility and Sterility, 2014, 102(1): 299-306.

[44] Jeong W, Jung S, Bazer F, et al. Stem cell factor-induced AKT cell signaling pathway: effects on porcine trophectoderm and uterine luminal epithelial cells. General and Comparative Endocrinology, 2017, 250: 113-121.

[45] Payer B, Saitou M, Barton SC, et al. Stella is a maternal effect gene required for normal early development in mice. Current Biology, 2003, 13(23): 2110-2117.

[46] Bendall SC, Hughes C, Campbell JL, et al. An enhanced mass spectrometry approach reveals human embryonic stem cell growth factors in culture. Molecular and Cellular Proteomics, 2009, 8(3): 421-432.

[47] Terskikh A, Bajpai R. Methods for culture and production of single cell populations of human embryonic stem cells (HESCS). Google Patents, 2017.

[48] Bahadorani M, Hosseini SM, Abedi P, et al. Glial cell line-derived neurotrophic factor in combination with insulin-like growth factor 1 and basic fibroblast growth factor promote in vitro culture of goat spermatogonial stem cells. Growth Factors, 2015, 33: 181-191.

[49] Wang H, Xiang J, Zhang W, et al. Induction of germ cell-like cells from porcine induced pluripotent stem cells. Scientific Reports, 2016, 6: 27256.

[50] Boozarpour S, Matin MM, Momeni-Moghaddam M, et al. Glial cell derived neurotrophic factor induces spermatogonial stem cell marker genes in chicken mesenchymal stem cells. Tissue and Cell, 2016, 48(3): 235–241.

[51] Kang L, Wang J, Zhang Y, et al. iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. Cell Stem Cell, 2009, 5: 135-138.

[52] Nayernia K, Lee J H, Drusenheimer N, et al. Derivation of male germ cells from bone marrow stem cells. Laboratory Investigation, 2006, 86(7): 654-663.

[53] Dyce P W, Wen L, Li J, et al. In vitro germline potential of stem cells derived from fetal porcine skin. Nature Cell Biology, 2006, 8(4): 384-390.

[54] Zhao X Y, Li W, Lü Z, et al. iPS cells produce viable mice through tetraploid complementation. Nature, 2009, 461(7260): 86-90.

[55] Kerkis A, Fonseca S A, Serafim R C, et al. In vitro differentiation of male mouse embryonic stem cells into presumptive sperm cells. Cloning and Stem Cells, 2007, 9(4): 535-548.

[56] Toyooka Y, Tsunekawa N, Akasu R, et al. Embryonic stem cells can form germ cells in vitro. Proceedings of the National Academy of Sciences, 2003, 100(20): 11457-11462.

[57] Bowles J, Koopman P. Retinoic acid, meiosis and germ cell development in mammals. Development, 2007, 134(19): 3401-3411.

[58] Bowles J, Knight D, Smith C, et al. Retinoid signaling determines germ cell fate in mice. Science, 2006, 312: 596-600.

[59] Pellegrini M, Grimaldi P, Rossi P, et al. Developmental expression of BMP4/ALK3/SMAD5 signalling pathway in the mouse testis: a role in spermatogonia differentiation. Journal of Cell Science, 2003, 116: 3363-3372.

[60] West FD, Roche-Rios M I, Abraham S, et al. KIT ligand and bone morphogenetic protein signaling enhances human embryonic stem cell to germ-like cell differentiation. Human Reproduction, 2010, 25(1): 168-178.

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