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REVIEW ARTICLE

Spermatogonial Stem Cells: A Review of Essential Factor for Self-Renewal and Differentiation

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Abstract

Introduction: Spermatogonial stem cells (SSCs) are considered as promising management for azoospermic patients who couldn't obtain sperms from the epididymis or directly from testis on assisted reproductive technologies (ART) procedure. The newt highlights of the current research are the emphasis on the utilization of several substances in SSCs culture system which could maintain pluripotency or induce differentiation of SSCs. The objective of this work was to systematically review and discuss articles dealing with the subject of essential factors for self-renewal and differentiation of SSCs. Methods: To this purpose, a computerized search of PubMed database was performed on the general term such as in vitro culture, spermatogonial stem cells, growth factor, hormone and antioxidant. Results: The addition of growth factor, hormone, and antioxidant each play a distinct role in proliferation and differentiation of SSCs. The supplementation of a growth factor activates the cascade of signal transduction toward self-renewal regulation. The addition of hormones such as FSH and testosterone or an antioxidant such as retinoic acid stimulates the differentiation of SSCs in vitro in a certain concentration. Conclusion: The mechanism of these substances in maintaining the pluripotency or inducing cell differentiation varies. Successful development of the SSCs culture system will encourage utilization of SSCs through in vitro proliferation and artificial sperm formation followed by auto-transplantation even though proper investigation is still required prior clinical application.

Keywords: *Spermatogonial Stem Cell, Growth Factor, Hormone, Antioxidant*

Introduction

Spermatogonial stem cells (SSC) are adult stem cells which have a vital role in spermatogenesis by providing sperm production throughout life in men [1, 2]. SSC reside in the basal layer of the seminiferous tubules surrounded by Sertoli cells that support spermatogenesis, providing matrix and nurturing the germ cells [3]. In the case of male infertility, there is disruption towards spermatogenesis process such as spermatogenic arrest which interrupts germ cells differentiation leading to severe oligozoospermia or even azoospermia. Currently, SSC are considered as a promising agent in order to manage male infertility particularly azoospermia.

The current studies are emphasis on the formation of artificial sperms in vitro further, which is further utilized in auto transplantation [4]. Therefore, research in the major development efforts of the SSC culture system will have enormous benefits in the future. The molecular mechanism of germ cell fate is high strictly regulated by intrinsic and extrinsic factors, either gene expression in SSCs or testicular niche factors [3]. Self-renewal and spermatogonia differentiation remarkably depend on several essential factors derived from Sertoli cells such as glial derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), and epidermal growth factor (EGF). A short and long-term culture of SSCs and utilization of growth factors that promote the requirements for in vitro propagation of SSC have been attempted in many researchs. Previous research demonstrated that SSC incubation in the presence of growth factors, hormones and antioxidants allows long-term

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proliferation in vitro and promotes differentiation in a certain concentrations. The objective of this study was to review the articles relating to the subject of growth factors that are essential for the development of SSC culture particularly underlying mechanism towards self-renewal and differentiation of SSC.

Methodology

A computerized search of PubMed database was performed to retrieve relevance data. The incorporation of the substance and the role in maintain proliferation and differentiation of SSC comprised of searching database and reference searches. On search, a combination of the key phrases and words such as in vitro culture, spermatogonial stem cells, growth factor, hormone, and antioxidant were used.

Results

Essential Factors for Self-Renewal and Differentiation Spermatogonial Stem Cells In Vitro

The current research of SSC culture is an emphasis on two models of culture systems which by supplementing growth factors to the media and providing a feeder layer of cells. Previous research demonstrated that combination of growth factors induces long-term survival of SSC for up to 6 months and maintains SSC differentiation [5]. Another research aimed to assess the appropriate substance which can be utilized in SSC culture about maintaining pluripotency or inducing differentiation as shown in Table 1. Furthermore, in this study, the author assessed the underlying mechanism towards self-renewal and differentiation of SSC which presented in Table 2.

Table 1: Essential factors to self-renewal and differentiation of SSC

Agent	Essential factors	Producing cells	Reference
Growth factor	Leukaemia inhibitory factor (LIF)	Peritubular, Sertoli, Leydig cells	[3, 6,7-11]
	Glial cell-derived neurotrophic factor (GDNF)	Sertoli cells	[6-10, 12, 13]
	Basic fibroblast growth factor (bFGF)	Sertoli cells	[6, 9, 10, 12-14]
	Epidermal growth factor (EGF)	Sertoli cells	[6, 7, 9, 10, 12, 13]
	Fibroblast growth factor (FGF) 2	Sertoli, Leydig, and germ cells	[15]
	Stem cell factor (SCF)	Sertoli cells	[16]
	Bone morphogenic factor (BMP) 4	Sertoli cells	[16]
	Colony stimulating factor (CSF) 1	Sertoli cells	[17]
Hormone	Follicle-stimulating hormone (FSH)	Pituitary	[18]
	Testosterone	Leydig cells	[18]
Antioxidant	Retinoic acid	Sertoli cells	[18, 19]

Table 2: The mechanism of essential factors to self-renewal and differentiation of SSC

Essential factors	Function	Mechanism of action	Reference
LIF	Survival and proliferation of primordial germ cells	Preventing apoptosis and activating JAK-Stat pathway	[3, 6,7-11]
GDNF	Inhibit SSC differentiation	Activating cascade of intracellular signaling such as phosphoinositide 3-kinase (PI3K) and Src family kinase (SFK)	[6-10, 12, 13]
bFGF	Inhibit SSC differentiation	Enhancing the influence of GDNF	[6, 9, 10, 12-14]
EGF	Regulation of germinal cells proliferation and differentiation	Enhancing the influence of GDNF	[6, 7, 9, 10, 12, 13]
FGF2	Promote proliferation of SSC	Activating mitogen-activated protein kinase pathways	[15]
SCF	Survival, proliferation and differentiation of SSC	SCF/c-kit systems enhance the regulation of cyclin D3 and cycle cell on spermatogonia through rapamycin-sensitive phosphoinositide 3-kinase/p70s6 kinase pathway.	[16]
BMP4	Promote the differentiation of SSC	Stimulate the expression of Smad1, Smad5, and Smad8 in the nucleus of SSC and blocking the SSC self-renewal	[16]
CSF1	Promote self-renewal and proliferation of SSC	Act in synergy and enhance the influence of GDNF	[17]
FSH	Survival of germinal cells, meiosis, spermiogenesis	Regulating the proliferation through GDNF/FSH pathway	[18]
Testosterone	Survival of germinal cells, meiosis, spermatid differentiation	Stimulating Sertoli cell function and germ cell differentiation	[18]
Retinoic acid	Promote the differentiation of SSCs	Downregulation of OCT4 and PLZF	[18, 19]

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Growth Factor Supplementation

Crucial factors for the proliferation and maintenance of SSCs both in vitro and in vivo are Glial cell-derived neurotrophic factor (GDNF). GDNF is a Sertoli cell-derived factor which controls the equilibrium of self-renewal or differentiation of the cell. In low concentration of GDNF, SSC tend to differentiate while in high concentration tending to maintain pluripotency. The ratio of either self-renewal or stem cell differentiation is highly regulated by GDNF. The receptor of GDNF, GFRA1, is expressed by SSC shows that spermatogonia responds to GDNF and Sertoli cells that are capable of regulating SSC quantities [16, 20].

GDNF binds to a complex receptor consisting of c-Ret tyrosin kinase and glycosylphosphatidylinositol (GPI)-anchored binding molecule GFRα1 (GDNF family receptor alpha 1). The interaction between ligand and receptor could activate the cascade of intracellular signaling such as phosphoinositide 3-kinase (PI3K) and Src family kinase (SFK) that induces Akt activation thus influence cell functions such as self-renewal (Fig.1). The SFK signaling pathway also induces specific gene expression essential for self-renewal of SSCs.

Transcription factors encoding several genes such as B cell CLL / lymphoma 6 (BCL6b), Ets variant gene 5 (ETV5) and Lim homeobox protein 1 (LHX1) are regulated through an important SFK activation pathway in self-renewal of SSC. PLZF and Taf4b factor transcription (TATA box-binding protein (TBP) which associated with Taf4b also has a role in SSC self-renewal [21]. However, GDNF does not influence the expression of PLZF or Taf4b in SSC culture. In addition, the GDNF-GFRA1 RET complex induces the expression of N-Myc through activation of Src and PI3K [22, 23].

GDNF plays a crucial role in regulating self-renewal of SSC, while basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) enhance the influence of GDNF, although it's underlying mechanism is undefined (Fig.1). The addition of bFGF alone is not adequate sufficient to produce similar results compared to the combination with GDNF [24]. Fibroblast growth factor (FGF) 2 is another extrinsic factor used in the SSC culture. FGF2 has a biological effect to promote proliferation through the MAP2K1 pathways. Transcription factors (Bcl6b, Etv5, and Lhx1) are important in self-renewal of SSC act downstream of the FGF2 pathway (Fig.1). In addition, MEK (mitogen-activated protein kinase/ERK1 kinase) activation is presented which likely to a downstream of FGF2 which is a key signal for the progression of cell cycle [15, 25]. Another Sertoli derived factor, stem cell factor (SCF) also known as KIT ligand, stimulates proliferation, differentiation and meiosis of SSC in vitro through c-kit receptor. In adult testis, not in SSC, Sertoli cells are stimulated by FSH express SCF ligand.

SCF/c-kit Systems enhances the regulation of cyclin D3 and cycle cells in spermatogonia through rapamycin-sensitive phosphoinositide 3-kinase/p70s6 kinase pathway. Mutation of c-Kit and SCF gene could influence spermatogenesis [15, 26]. Bone morphogenetic protein (BMP) 4 promotes SSC differentiation in vitro through stimulation of Smad1, Smad5, and Smad8 expression in the nucleus of SSC and inhibits SSC self-renewal. Oatley *et al* (2009) suggested that the addition of a recombinant colony stimulating factor 1 (CSF1) to a culture media enhances the ability of SSC to self-renewal in heterogeneous Thy+1 spermatogonial culture. CSF1 through its receptor, CSF1r, along with GDNF and FGF2 enhances the pluripotency of Thy+1 germ cells .In the agreement, CSF1, produced by Leydig and myoid cells, acts in synergy with the GDNF that promotes SSCs self-renewal [17] .Leukaemia inhibitory factor (LIF) is a 38-67 kDa polypeptide cytokine which belongs to the IL-6 family with various variables of biological actions in different tissue systems [27].

In a study, it was reported that LIF is an essential component for long-term culture of SSC by preventing apoptosis. LIF binds to a receptor complex comprising of two transmembrane proteins, LIFR and GP130 [24]. The interaction of LIF to its receptor promotes activation of two major intracellular signaling pathways, JAK-Stat pathway and SHP2-ERK pathway. The binding of LIF to its receptor triggers the activation of Janus-associated tyrosine kinase (JAK) to further phosphorylate STAT3 that promotes dimerization of STAT3. STAT3 dimers are translocated to the nucleus where they bind to sites on DNA that

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control important gene transcription in stem cells self-renewal. On the other hand, LIF could promote the effect of differentiation by stimulating gp130. Recent studies have demonstrated that its combination with bFGF elicits a much higher effect toward in vitro SSC expansion.

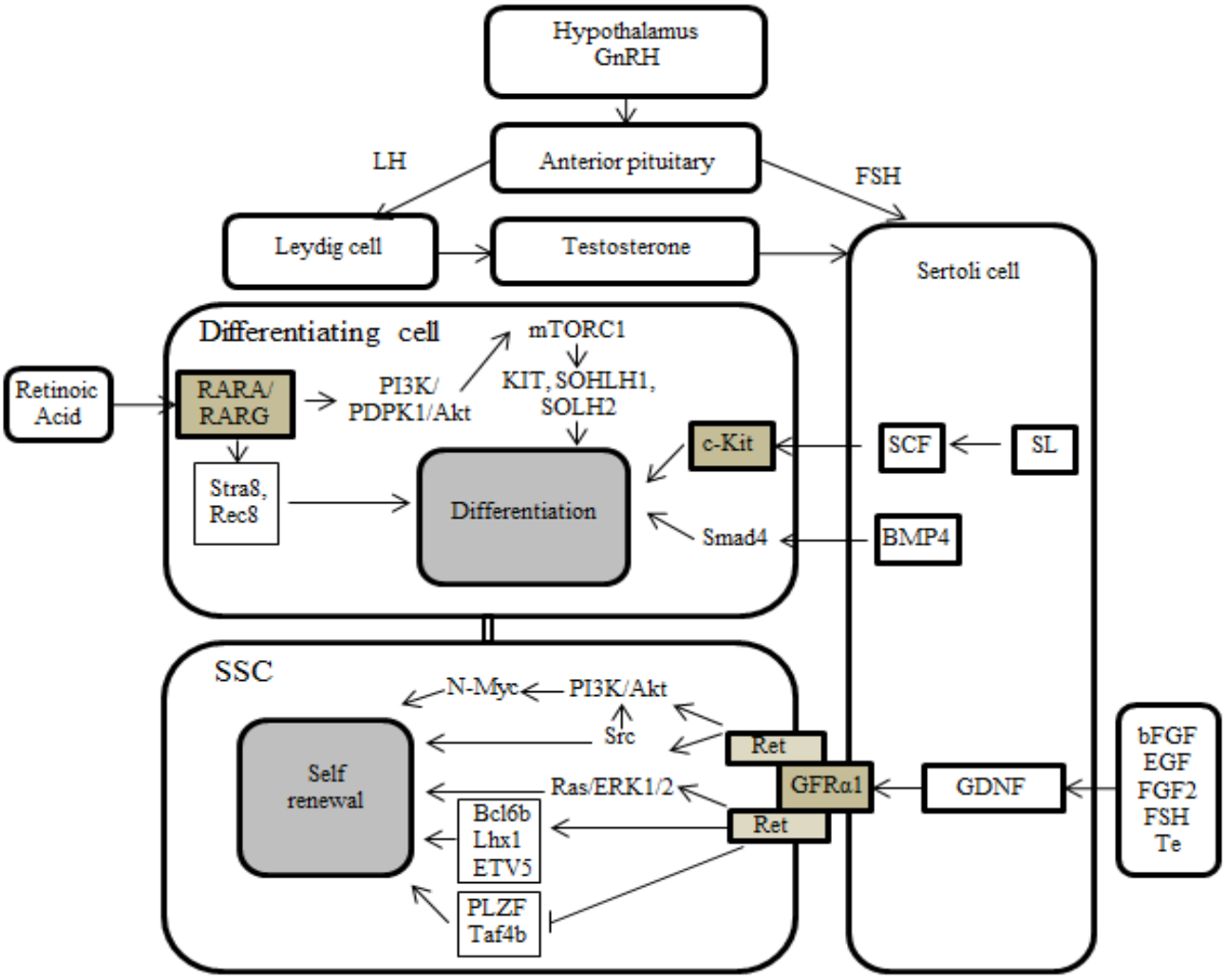

Fig. 1: A schematic representation of signaling model towards self-renewal and differentiation of SSC. BCL6b: B cell CLL/lymphoma 6; bFGF: Basic fibroblast growth factor; EGF: Epidermal growth factor; ETV5: Ets variant gene 5; GDNF: Glial cell-derived neurotrophic factor; GnRh: gonadotropin-releasing hormone; FGF2: Fibroblast growth factor 2; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; LHX1: Lim homeobox protein 1; PDPK1: 3-Phosphoinositide Dependent Protein Kinase 1; PI3K: Phosphoinositide 3-kinase; RAR: Retinoic acid receptor (A/G); SL: Steel; SCF: Stem cell factor; SSC: Spermatogonial stem cells; Te: Testosterone

Hormone Supplementation

Endocrine stimulation in spermatogenesis involves follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, produced by Leydig cells in the testis. Action testosterone and FSH stimulate synergistic effects on meiotic and postmeiotic germ cells in vivo and in vitro. FSH and testosterone have pivotal role and prerequisites for the completion of meiosis and spermiogenesis in vitro. The presence of FSH and testosterone acts as a survival factor by preventing apoptosis and inducing in meiosis and spermatid differentiation. The addition of testosterone in vitro stimulates Sertoli cell function and germ cell differentiation to the haploid stage [18]. Sertoli cell express FSH and testosterone receptor, so as to regulate proliferation and differentiation of SSC through GDNF/FSH pathway (Fig.1).

Antioxidant Supplementation

Differentiation of SSC involves retinoic acid (RA), SCF/c-kit Systems, cyclin D2 and D3, DAZL protein [28]. Sertoli cells regulate the formation and degradation of RA through the expression of RA-metabolizing enzymes, Cyp26b1 which able to degrade RA following act upon the germ cells [29]. RA triggers spermatogonial differentiation through direct or indirect down regulation of OCT4 and PLZF [30]. RA regulates the ultimate function of the cell by binding RARs to the RA response element (RAREs) on the target gene promoter. Busada, *et al* (2016) described

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that RARA is bound to the regulatory subunit (p85) of PI3K, recruits the catalytic subunit (p110), and promotes the phosphorylation of ERK and AKT [19]. In agreement, RA is capable to generate activation of PI3K/PDK1/AKT/mTORC1 signaling pathway that encourage the translation of represses mRNAs, such as Kit, Sohlh1, Sohlh 2.

The cascade activation of the PI3K/AKT signaling pathway followed by the binding of KITL to the tyrosine kinase receptor is important for spermatogonial development. In addition, signals generated by PI3K and AKT, which induce proliferation and differentiation signals, impinge the regulation of protein synthesis through mTORC1 activation. Together, the RA signals through PI3K/AKT pathways that in turn activates mTORC1 supports spermatogonial differentiation [19].

Conclusion

The findings performed that the supplementation substances such as growth factors, antioxidants and hormones could be used in SSC culture in order to increase the quantity or promote the differentiation of SSC .Utilization of growth factors that promote propagation and long-term culture of SSC in particular concentration. The addition of FSH, testosterone and retinoic acid induce differentiation of SSC. The mechanisms of these substances in maintaining pluripotency or inducing cell differentiation of cells vary. Surely, the utilization of variance substances in culture systems should be further investigated prior to clinical application.

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Ethics

This review article is original and contains unpublished material. The corresponding author confirms that all of the authors have read and approved the manuscript and no ethical issues involved.

References

1. Vassena R, Eguizabal C, Heindryckx B, Sermon K, Simon C, van Pelt AM, et al (2015) Stem cells in reproductive medicine: ready for the patient? Hum Reprod. 30(9):2014-21.
2. Yang QE, Oatley JM (2014) Spermatogonial stem cell functions in physiological and pathological conditions. Curr Top Dev Biol. 107:235-67.
3. Rastegar T, Roudkenar MH, Parvari S, Baazm M (2015) The interaction between sertoli cells and luekimia inhibitory factor on the propagation and differentiation of spermatogonial stem cells in vitro. Iran J Reprod Med. 13(11):679-86.
4. Lestari SW, Aditya D (2017) Spermatogonial Stem Cells: A Current Update in the Management of Azoospermic Infertile Men. OnLine Journal of Biological Sciences. 17(4):353-8.
5. Liu S, Tang Z, Xiong T, Tang W (2011) Isolation and charaterization of human spermatogonial stem cells. Reproductive Biology and Endocrinology. 9(141).
6. Koruji M, Shahverdi A, Janan A, Piryaei A, Lakpour MR, Gilani Sedighi MA (2012) Proliferation of small number of human spermatogonial stem cells obtained from azoospermic patients. J Assist Reprod Genet. 29(9):957-67.
7. Akhondi MM, Mohazzab A, Jeddi-Tehrani M, Sadeghi MR, Eidi A, Khodadadi A, et al (2013) Propagation of human germ stem cells in long-term culture. Iran J Reprod Med. 11(7):551-8.
8. Sadri-Ardekani H, Mizrak SC, van Daalen SKM, Korver CM, Roepers-Gajadien HL, Koruji M, et al (2009) Propagation of human Spermatogonial Stem Cells in vitro. JAMA. 302(19):2127-34.
9. Goharbakhsh L, Mohazzab A, Salehkhou S, Heidari M, Zarnani AH, Parivar K, et al (2013) Isolation and culture of human spermatogonial stem cells derived from testis biopsy. Avicenna J Med Biotechnol. 5(1):54-61.
10. Guo Y, Hai Y, Gong Y, Li Z, He Z (2014) Characterization, isolation, and culture of mouse and human spermatogonial stem cells. J Cell Physiol. 229(4):407-13.
11. Lim JJ, Sung SY, Kim HJ, Song SH, Hong JY, Yoon TK, et al (2010) Long-term

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proliferation and characterization of human spermatogonial stem cells obtained from obstructive and non-obstructive azoospermia under exogenous feeder-free culture conditions. Cell Prolif. 43(4):405-17.

12. Choi NY, Park YS, Ryu JS, Lee HJ, Arauzo-Bravo MJ, Ko K, et al (2014) A novel feeder-free culture system for expansion of mouse spermatogonial stem cells. Mol Cells. 37(6):473-9.
13. Lim JJ, Seol DW, Choi KH, Shin DH, Kim HJ, Song SH, et al (2014) Spermatogonial stem cell enrichment using simple grafting of testis and in vitro cultivation. Sci Rep. 4, 5923.
14. Wahab A, Lokman M, Ramli R (2016) Spermatogonial stem cells protein identification in vitro culture from non-obstructive azoospermia patient. Malays J Med Sci. 23(3):40-8.
15. Mei XX, Wang J, Wu J (2015) Extrinsic and intrinsic factors controlling spermatogonial stem cell self-renewal and differentiation. Asian J Androl. 17(3):347-54.
16. Singh SR, Burnicka-Turek O, Chauhan C, Hou SX(2011) Spermatogonial stem cells, infertility and testicular cancer. J Cell Mol Med. 15(3):468-83.
17. Oatley JM, Oatley MJ, Avarbock MR, Tobias JW, Brinster RL (2009) Colony stimulating factor 1 is an extrinsic stimulator of mouse spermatogonial stem cell self-renewal. Development. 136(7):1191-9.
18. Zanganeh BM, Rastegar T, Roudkenar MH, Kashani IR, Amidi F, Abolhasani F, et al (2013) Co-culture of spermatogonial stem celss with sertoli cells in the presence of testosterone and FSH improved differentiation via up-regulation of post meiotic genes. Acta Medica Iranica. 51(1).
19. Busada JT, Geyer CB (2016) The role of retinoic acid (RA) in spermatogonial differentiation. Biology of Reproduction. 94(1):1-10.
20. Komeya M, Ogawa T (2015) Spermatogonial stem cells: Progress and prospects. Asian J Androl. 17(5):771-5.
21. Lovasco LA, Gustafson EA, Seymour KA, de Rooij DG, Freiman RN (2015) TAF4b is Required for Mouse Spermatogonial Stem Cell Development. Stem Cells. 33(4):1267-76.
22. Braydich-Stolle L, Kostereva N, Dym M, Hofmann MC (2007) Role of Src family kinases and N-Myc in spermatogonial stem cell proliferation. Dev Biol. 304(1):34-45.
23. Hofmann MC (2008) Gdnf signaling pathways within the mammalian spermatogonial stem cell niche. Mol Cell Endocrinol. 288(1-2):95-103.
24. Oatley JM, Brinster RL (2008) Regulation of spermatogonial stem cell self-renewal in mammals. Annu Rev Cell Dev Biol. 24:263-86.
25. Simon L, Ekman GC, Tyagi G, Hess RA, Murphy KM, Cooke PS (2007) Common and distinct factors regulate expression of mRNA for ETV5 and GDNF, Sertoli cell proteins essential for spermatogonial stem cell maintenance. Exp Cell Res. 313(14):3090-9.
26. Nayernia K, Nolte J, Michelmann HW, Lee JH, Rathsack K, Drusenheimer N, et al (2006) In vitro-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. Dev Cell. 11(1):125-32.
27. Huleihel M, Abuelhija M, Lunenfeld E (2007) In vitro culture of testicular germ cells: regulatory factors and limitations. Growth Factors. 25(4):236-52.
28. de Kretser DM, Loveland K, O'Bryan M (2016) Spermatogenesis. Endocrinology : Adult & Pediatric. 7: Elsevier; 2325-53.e9.
29. Wang S, Wang X, Ma L, Lin X, Zhang D, Li Z, et al (2016) Retinoic acid is sufficient for the in vitro induction of mouse spermatocyte. Stem Cell Reports. 7:80-94.
30. Dann CT, Alvarado AL, Molyneux L, Denard BS, Garbers DL, Porteus MH (2008) Spermatogonial Stem Cell Self-Renewal Requires OCT4, a Factor Downregulated During Retinoic Acid-Induced Differentiation. Stem Cells. 26:2928-37.

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