Int J Reprod BioMed Vol. 15. No. 5. pp: 265-272, May 2017

Review articel

Protective effects of melatonin and ghrelin on spermatogenesis: A narrative review of the literature

Mohammadreza Gholami[1] Ph.D., Seyyed Amir Yasin Ahmadi[2] M.D. Student., Abolfazl Abaszadeh[3] M.D., Arash Khaki[4] D.V.M., Ph.D.

1. *Department of Anatomy, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.*
2. *Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran.*
3. *Department of Surgery, Lorestan University of Medical Sciences, Khorramabad, Iran.*
4. *Department of Veterinary Pathology, Islamic Azad University, Tabriz Branch, Tabriz, Iran.*

Mohammadreza Gholami and Seyyed Amir Yasin Ahmadi are an equal first author.

Corresponding Author:
Arash Khaki, Department of Veterinary Pathology, Islamic Azad University, Tabriz Branch, Tabriz, Iran.
Email: khaki@iaut.ac.ir
Tel: (+98) 9143138399

Received: 6 September 2016
Revised: 8 January 2017
Accepted: 22 May 2017

Abstract

Spermatocytogenesis starts from lumens of seminiferous cords and after migration to the basal membrane ends to the lumens again. We attempt to review the protective effects of melatonin and ghrelin on Spermatocytogenesis and in particular on spermatogonial stem cells, as two rather newly-discovered hormones. Testicular freezing prior to chemotherapy and radiotherapy is one of the ways of preserving fertility in children with cancer. The freezing has two methods of slow-freezing (cryopreservation) and rapid-freezing (vitrification). Administration of melatonin can maintain the quality of the germ cells underwent such processes, as well as ghrelin, can protect germ cells from the toxicities secondary to ischemic injuries, and pathologic apoptosis. This review indicates that in vitro or in vivo administration of melatonin or ghrelin, could be effective to preserve fertilization and also they can be used in assisted reproductive technologies to improve the quality of sperms. Future original studies should be propelled toward human studies, of course with observing the ethics.

Key words: *Spermatogonia, Cryopreservation, Vitrification, Melatonin, Ghrelin.*

Introduction

Primordial germ cells (PGCs) are known as the origin of germ cells in both genders (1). However, most of the related studies have been done on mice. The origin of PGCs is still controversial, but it seems that they originate from an epiblastic tissue out of gonad and then migrate toward primordial genital ridge (2, 3). Development of PGCs is controlled by bone-morphometric proteins like the bone morphogenic protein-4 secreted by extra-embryonic ectoderm (4-6). In males, upon PGCs get to genital crest, they are surrounded by Sertoli cells and seminiferous cords are formed (7-9). At this time, the PGCs influenced by Notch signaling pathway of the Sertoli cells, are called as gonocyte (8, 10).

The gonocytes keep on proliferation till arresting phase after the embryonic day 14.5 (in mice) (10). After birthday, the gonocytes move from lumen of the cords to the basal membrane (11). During the motion, the proliferation starts again and spermatogonium-A (SA) is formed. After that, the SAs get away from the basal membrane to the lumen again and during the motion, they create respectively intermediate spermatogonium and spermatogonium-B (SB) (12). Briefly, spermatocytogenesis stars from lumens of the cords and after migration to the basal membrane ends to the lumens again (Figure 1).

SA formation, in turn, includes three levels; the A single spermatogonium (A_s) which also is called as spermatogonial stem cell (SSC); and the next levels are respectively A paired spermatogonium (A_p) and A aligned spermatogonium (A_{al}) (12). Spermatogonial stem cells (SSCs) maintain their population through self-renewal (10, 13). The self-renewal can maintain the balance existing between A_s and A_p. The ability to self-renewal is due to the fact that SSCs are undifferentiated like other stem cells (14, 15).

The gene *inhibitor of DNA binding 4* (*ID4*) is the involving gene in the mentioned balance. Reduction in expression of this gene is along with the reduction of SSC proliferation and infertility. The protein NANOS2 existing in A_s and A_p, is also involved that its elimination or over-expression cause elimination or aggregation of SSCs (16).

The inducing agent of SSC proliferation is glial cell line-derived neurotrophic factor (GDNF) and its signaling pathway released from Sertoli cells. Over-expression of this cascade results in aggregation of undifferentiated spermatogonia. Gradual elimination of SSCs could be secondary to either elimination of the GDNF or its receptors (17). Formation of intermediate spermatogonia and SBs is due to the protein spermatogenesis and oogenesis specific helix-loop-helix (SOHLH) (18).

Spermatogenesis injuries could be due to the toxicity of chemotherapeutic drugs such as busulfan and cispelatin or toxicity of antibiotics like gentamicin, infectious agents like parasites, the oxidative stress modeled by ischemia-reperfusion in studies or exposing in electromagnetic fields (19-30). Previously some antioxidants in onion juice and watermelon extract or other herbal medicines have been investigated as improvers of sperm parameters in such conditions (31, 32).

Testicular freezing prior to chemotherapy (with medicines like cisplatin, busulfan or cyclophosphamide) and radiotherapy is of the ways of preserving fertility in children with cancer (19, 21, 33, 34). The freezing has two methods of slow-freezing (cryopreservation) and rapid-freezing (vitrification) (34-36). In order to perform these technics, it seems better to use SSCs instead of spermatozoa; because in patients who has not reached the puberty age, we do not have any spermatozoon, and also SSCs are not differentiated enough to have acrosomal vesicles, so they have lower metabolic activity and hence they are lower at exposed to abnormalities. However, the freezing-thawing process could result in damage and quality reduction of the cells (34).

Melatonin has been found and proven in microorganisms such as primitive bacteria and green algae as a serve antioxidant.In addition, melatonin is also found in pluricellular organisms such as fungi, plants, insects, nematodes, and vertebrates like mammals (37). This hormone is secreted by the pineal gland and testes (38). Other than melatonin, ghrelin is a 28-amino acid endocrine peptide which is principally produced by the stomach (25-27, 39). Indeed ghrelin is a gut-brain hormone increasing food intake, inducing hunger and appetite via hypothalamic circuits. As well, this hormone causes the release of growth hormone and also induces adiposity in animal studies. In animal studies, central and peripheral administration of ghrelin activates the mesolimbic dopamine system mediating behaviors (40). Some new evidence highlight its role in reproductive functions regulation. In other words, this protein is not merely limited to gut and brain and has been detected previously in the tubular and interstitial compartments of testes of several vertebrate species (39).

After the narrative introduction presented above, we attempt to review the protective effects of melatonin and ghrelin on male reproduction system and in particular on SSCs, as two rather newly-discovered hormones.

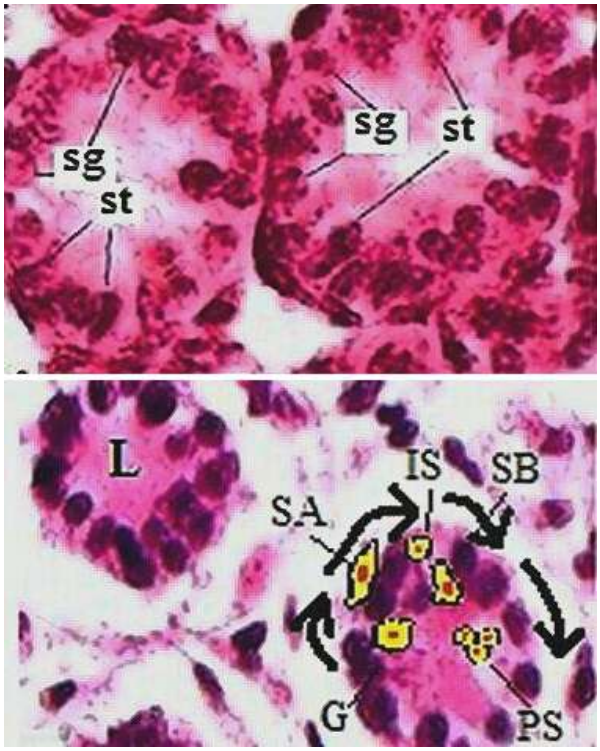

Figure 1. Spermatocytogenesis from the lumen to the lumen. L (lumen), PS (primary spermatocyte), G (gonocyte), SA (spermatogonia A), SB (spermatogonia B), IS (intermediate spermatogonia), sg (spermatogonia) and st (Sertoli cells) . The arrowed cells in yellow color, have been added graphically and schematically. Magnification ×400.

Materials and methods

For the present narrative review, we used the scientific databases and meta-search engines including Google Scholar, PubMed, Scopus and Science Direct, and Web of Science core collection. Since the related articles were rare, the time period was not important to us. As key words and contents of search, at first we searched for all the articles of the first and corresponding authors related to male's reproduction as the self-referring which is necessary for review articles; second, searched for "melatonin AND spermatogonia" and "ghrelin AND sperm"; and finally searched for general information about spermatogenesis process in order to write the introduction. There were some duplications between the first and the second levels. Our literature review method was summery-comparison matrix, a method helping researchers to extract relevant information from the literature, categorize and visualize them (41).

Results

The founded literatures are summarized in table I. The rows are sorted by time from the oldest to the newest. The results are from 1999 to 2016. Investigation of ghrelin on male reproduction system is a more emerging idea. These findings are discussed as below.

Table I. The literature review matrix

Hormone	Author, date (reference)	Main findings
Melatonin		
	Badr et al, 1999 (60)	Single-dose injection of melatonin to mice, before irradiation, could preserve the fertility.
	d'Istria et al, 2003 (71)	Melatonin shows anti-proliferative effect in frags.
	Ghasemi et al, 2010 (68)	Melatonin may have a protective effect on busulfan induced testicular damage.
	Hemadi et al, 2009,11 (73,74)	Melatonin after thawing the vitrified tissue can lead to better maintenance of spermatogenesis kinetics and it may serve as a promising method for graft preservation.
	Gholami et al, 2013 (34)	The sertoli cell modeling of seminiferous tubes induced by 40 mg/kg busulfan, shows that melatonin can induce spermatogenesis. Melatonin induce apoptosis in the damaged cells underwent freezing-thawing process. Hence it can be used for cell screening during ART* process.
	Yang et al, 2014 (70)	This bovine study shows that melatonin has anti-apoptotic effects through up-regulation of spermatogenesis-related genes like *cyclin D1* and *cyclin E*
	Gholami et al, 2014,15 (38,69)	Melatonin improves efficacy of SSC transplantation.
	Saki et al, 2016 (72)	In spite of the advantages of melatonin, it cannot prevent release of lactate dehydrogenase.
	Deng et al, 2016 (61)	Administration of melatonin results in differentiation of SSCs to haploid germ cells in vitro, other than the advantages mentioned before.
Ghrelin		
	Tena-Sempere, 2005 (75)	Expression of ghrelin was observed in Leydig cells and also its receptor, the growth hormone secretagogue receptor (GHSR) type 1a was observed in both Leydig and Sertoli cells.
	Olejniczak et al, 2009 (76)	Ghrelin dose-dependently inhibit testosterone secretion in vitro and modulate proliferation of leydig cells in vivo, as well as the expression of *encoding stem cell factor* and other testis-related genes. The expression site of ghrelin in seminiferous tubes can indicate the role of its in local regulation of spermatogenesis.
	Garcia et al, 2015 (78)	Ghrelin can prevent the sequels of cisplatin.
	Whirledge et al, 2015 (77)	Ghrelin can prevent the sequels of cisplatin. The receptor of ghrelin, the very GHSR type 1ahas also an important role. Thus it seems that GHSR agonists could be used for prevention from cisplatin-induced testicular damage.
	Taati et al, 2016 (25)	Ghrelin can improve sperm quality in the testes underwent ischemia-reperfusion injury.

The findings are currently based on animal studies
* ART: assisted reproductive technology

Discussion

Role of melatonin

There are some ways introduced to maintain quality of the germ cells undergoing freezing-thawing process and also the quality of sperms and other germ cells; for instance, antioxidants such as Na_2SeO_3, quersetine or other flavonoid compounds, magnesium sulfate or antioxidants in medical plants like ginger and onion, carrot seed extract (56) or water melon seed, chemical drugs like tramadol and adding vitamins C and/or D in vitro (32, 42-58). Using melatonin is one of these methods.The quality of germ cells and sperms could be assayed by proteomic technics (59).

The history of using melatonin dates back to the year 1999 when Badr, Habit and Harraz showed that single-dose injection of melatonin to mice, before irradiation, could preserve the fertility (60). From then on, there have been a

few types of research in this application of its. The most recent study, shows that administration of melatonin results in differentiation of SSCs to haploid germ cells in vitro, other than the advantages mentioned before (61). Gholami and coworkers had shown this advantage through the Sertoli cell modeling of seminiferous tubes induced by 40 mg/kg busulfan (62).

Melatonin is secreted by the pineal gland and testes (38). The fascinating feature of this hormone is that it makes the normal cells proliferate and makes the cancerous cells undergo apoptosis (62). Gholami *et al* showed that melatonin in mice induces apoptosis in the damaged cells underwent a freezing-thawing process (62). Although at the first, glans apoptosis has a negative connotation, hereby the cells are screened. This induction was through up-regulation of Fas and down-regulation of P53. Apoptosis is the most famous type of programmed cell death which is often physiologic and necessary like during implantation process of an embryo and sometimes is pathologic like secondary to a spinal cord trauma (63-67). Another example of physiologic apoptosis is maintaining the balance between the number of SSCs and gametes (68). Melatonin also improves the efficacy of SSC transplantation (38). Histopathologic studies show that melatonin reduces the oxidative injury to seminiferous tubes secondary to transplantation process in sheep (69).

However, there are some controversies. For example, a bovine study shows that melatonin has anti-apoptotic effects through up-regulation of spermatogenesis-related genes like *cyclin D1* and *cyclin E*, while another study shows anti-proliferative effect of melatonin in frogs (70, 71). Saki and the coworkers believe that in spite of the advantages of melatonin, it cannot prevent the release of lactate dehydrogenase (72). Moreover, Ghasemi *et al* believed that melatonin could have a protective effect on busulfan-induced testicular damage (68). Hemadi and the coworkers believe that use of melatonin after thawing the vitrified tissue can lead to better maintenance of spermatogenesis kinetics and it may serve as a promising method for graft preservation (73, 74).

Role of ghrelin

In 2005, expression of ghrelin was observed in Leydig cells and also its receptor, the growth hormone secretagogue receptor (GHSR) type 1a was observed in both Leydig and Sertoli cells (75). In 2009 it has been shown that the expression site of ghrelin in seminiferous tubes can indicate the role of its in local regulation of spermatogenesis (76). This hormone has been proven to dose-dependently inhibit testosterone secretion in vitro, and modulate proliferation of Leydig cells in vivo, as well as the expression of encoding stem cell factor and other testis-related genes (75).

Cisplatin is an anti-cancer medicine which induces apoptosis both in cancerous and normal cells (20, 21). The apoptotic activity of cisplatin is due to inhibition of P53-dependent DNA repair. Among the sequels of cisplatin, damage to the germ cells can be pointed out; for example, damage to SSCs. It has been observed that ghrelin can prevent this sequel (77, 78). The receptor of ghrelin, the very GHSR type 1ahas also an important role. Thus it seems that GHSR agonists could be used for prevention from cisplatin-induced testicular damage (77).

In addition to cisplatin-induced damage, the studies done by Taati and the coworkers during recent years, show the protective effects of ghrelin from ischemia-reperfusion damage. They found that malondialdehyde values were significantly lowered in the treated group, and ghrelin significantly enhanced sperm movement, motility, and concentration. This protective effect is due to anti-apoptotic and anti-inflammatory effects of ghrelin (25-27). It seems that the similar feature of melatonin and ghrelin is their inhibitory role in mammalian reproduction.

Conclusion

Infertility is a problem for a lot of couples. The male-based ones, either in real clinics or

in research modeling, could be due to chemotherapy, radiotherapy, electromagnetic fields, ischemia-reperfusion and high oxidative stress, microbial and parasitic agents, or toxicity of antibiotics. This review indicates that in vitro or in vivo administration of melatonin and ghrelin as two newly discovered hormones, could be effective to preserve fertilization in such cases, and also they can be used in assisted reproductive technologies to improve the quality of the sperms. Future original studies should be propelled toward human studies of course with observing the ethics.

Conflict of interest

Hereby we declare that there is no conflict of interest.

References

1. Brieño-Enríquez MA, García-López J, Cárdenas DB, Guibert S, Cleroux E, Děd L, et al. Exposure to endocrine disruptor induces transgenerational epigenetic deregulation of microRNAs in primordial germ cells. *PloS One* 2015; 10: e0124296.
2. Montiel-Eulefi E, Sanchez R, Rojas M, Bustos-Obregon E, Montiel-eulefi E, Sánchez R, et al. Epiblast embryo stem cells give origin to adult pluripotent cell populations: primordial germ cell, pericytic and haematopoyetic stem cells. A Review. *Int J Morphol* 2009; 27: 1325-1333.
3. Qiao J-l, Zhao E-f, Peng H-m. The origin, migration, proliferation and diffrentiation of human primordial germ cell. *Prog Anat Sci* 2012; 1: 024.
4. Rezaei M, Karbalaie K, Tanhaie S, Madani H, Nasr Esfahani MH, Baharvand H. Bone morphogenetic protein-4 influences neural differentiation of induced mouse mesenchymal stem cells. *Yakhteh* 2011; 12: 511-516.
5. Nakamura T, Extavour CG. The transcriptional repressor Blimp-1 acts downstream of BMP signaling to generate primordial germ cells in the cricket Gryllus bimaculatus. *Development* 2016; 143: 255-263.
6. Owchi MA, Salehnia M, Moghadam MF, Boroujeni MB, Hajizadeh E. The effect of bone morphogenetic protein 4 on the differentiation of mouse embryonic stem cell to erythroid lineage in serum free and serum supplemented media. *Int J Biomed Sci* 2009; 5: 275-282.
7. Petersen AM, Earp NC, Redmond ME, Postlethwait JH, von Hippel FA, Buck CL, et al. Perchlorate Exposure Reduces Primordial Germ Cell Number in Female Threespine Stickleback. *PLoS One* 2016; 11: e0157792.
8. Sakashita A, Kawabata Y, Jincho Y, Tajima S, Kumamoto S, Kobayashi H, et al. Sex Specification and Heterogeneity of Primordial Germ Cells in Mice. *PloS One* 2015; 10: e0144836.
9. Kjartansdóttir KR, Reda A, Panula S, Day K, Hultenby K, Söder O, et al. A Combination of Culture Conditions and Gene Expression Analysis Can Be Used to Investigate and Predict hES Cell Differentiation Potential towards Male Gonadal Cells. *PloS One* 2015; 10: e0144029.
10. Garcia TX, Hofmann M-C. NOTCH signaling in Sertoli cells regulates gonocyte fate. *Cell Cycle* 2013; 12: 2538-2545.
11. Mahla RS, Reddy N, Goel S. Spermatogonial stem cells (SSCs) in buffalo (Bubalus bubalis) testis. *PLoS One* 2012; 7: e36020.
12. Okuda H, Kiuchi H, Takao T, Miyagawa Y, Tsujimura A, Nonomura N, et al. A Novel Transcriptional Factor Nkapl Is a Germ Cell-Specific Suppressor of Notch Signaling and Is Indispensable for Spermatogenesis. *PloS One* 2015; 10: e0124293.
13. Bahadorani M, Hosseini SM, Abedi P, Hajian M, Hosseini SE, Vahdati A, et al. Short-term in-vitro culture of goat enriched spermatogonial stem cells using different serum concentrations. *J Assist Reprod Genet* 2012; 29: 39-46.
14. Safarinejad MR. Editorial comment to: Recruiting Testicular Torsion Introduces an Azoospermic Mouse Model for Spermatogonial Stem Cell Transplantation. *Urol J* 2014; 11: 1656.
15. Boroujeni MB, Salehnia M, Valojerdi MR, Moghadam MF. Transplantation and homing of mouse embryonic stem cells treated with erythropoietin in spleen and liver of irradiated mice. *Iran Biomed J* 2009; 13: 87-94.
16. Ferguson L, How JJ, Agoulnik AI. The fate of spermatogonial stem cells in the cryptorchid testes of RXFP2 deficient mice. *PloS One* 2013; 8: e77351.
17. Dovere L, Fera S, Grasso M, Lamberti D, Gargioli C, Muciaccia B, et al. The niche-derived glial cell line-derived neurotrophic factor (GDNF) induces migration of mouse spermatogonial stem/progenitor cells. *PloS One* 2013; 8: e59431.
18. Zhang X, Liu R, Su Z, Zhang Y, Zhang W, Liu X, et al. Immunohistochemical Study of Expression of Sohlh1 and Sohlh2 in Normal Adult Human Tissues. *PloS One* 2015; 10: e0137431.
19. Ahar NH, Khaki A, Akbari G, Novin MG. The effect of Busulfan on body weight, Testis weight and MDA enzymes in male rats. *Int J Women's Health Reprod Sci* 2014; 2: 316-319.
20. Baradaran A, Tavafi M, Ardalan M-R, Rafieian-Kopaei M. Cisplatin; nephrotoxicity and beyond. *Annal Res Antioxidant* 2016; 1: e14.
21. Shahsavar F, Bozorgmehr M, Mirzadegan E, Abedi A, Lighvan ZM, Mohammadi F, et al. A novel platinum-based compound with preferential cytotoxic activity against a panel of cancer cell lines. *Anti-Cancer Agents Med Chem* 2016; 16: 393-403.
22. Zahedi A, Khaki A, Ahmadi-Ashtiani H, Rastegar H, Rezazadeh S. Zingiber officinale protective effects on gentamicin's toxicity on sperm in rats. *J Med Plants* 2010; 3: 93-98.

23. Kalpana T. Effect of gentamicin and role of antioxidant on spermatogenesis in albino rats. *J Anat Soc India* 2016; 65: S87.
24. Talebi Farahani M, Hoseini F, Minai-Tehrani A, Novin MG. The effect of infection with genital Mycoplasma hominis and the presence of antisperm antibodies in Iranian women with unexplained infertility. *Int J Women's Health Reprod Sci* 2016; 4: 18-22.
25. Taati M, Moghadasi M, Dezfoulian O, Asadian P. Effects of Ghrelin on Testicular Ischemia/ Reperfusion-Induced Injury. *Acta Medica Iranica* 2016; 54: 32-38.
26. Taati M, Moghadasi M, Dezfoulian O, Asadian P, Kheradmand A, Abbasi M, et al. The effect of ghrelin pretreatment on epididymal sperm quality and tissue antioxidant enzyme activities after testicular ischemia/ reperfusion in rats. *J Physiol Biochem* 2012; 68: 91-97.
27. Taati M, Moghadasi M, Dezfoulian O, Asadian P, Zendehdel M. Effects of Ghrelin on germ cell apoptosis and proinflammatory cytokines production in Ischemia-reperfusion of the rat testis. *Iran J Reprod Med* 2015; 13: 85-92.
28. Asghari A, Montaseri A, Khaki A. An Ultrastructural Study of the Antioxidant Effects of VitaminE and Fennel Extract on Zona Pellucida Cell Changes of RatOvaries under Non-Ionizing 50Hz Electromagnetic Fields. *Crescent J Med Biol Sci* 2015; 2: 37-41.
29. Asghari A, Montaseri A, Khaki A. A Study of the Protective Effects of Vitamin E and FennelExtract on Mitochondria Changes in Mice Ovary Due toElectromagnetic Field Exposure. *Crescent J Med Biol Sci* 2015; 2: 10-13.
30. Khaki A, Ali -Hemmati A, Nobahari R. A Study of the Effects of Electromagnetic Field on Islets ofLangerhans and Insulin Release in Rats. *Crescent J Med Biol Sci* 2015; 2: 1-5.
31. Garedaghi Y, Bahavarnia SR. Repairing effect of allium cepa on testis degeneration caused by toxoplasma gondii in the rat. *Int J Women's Health Reprod Sci* 2014; 2: 81-89.
32. Khaki A, Fathiazad F, Nouri M. Effects of watermelon seed extract (citrullus vulgaris) on spermatogenesis in rat. *Int J Women's Health Reprod Sci* 2013; 1: 99-104.
33. Jalali AS, Hasanzadeh S, Malekinejad H. Crataegus monogyna aqueous extract ameliorates cyclophosphamide-induced toxicity in rat testis: stereological evidences. *Acta Medica Iranica* 2012; 50: 1.
34. Gholami M, Hemadi M, Saki G, Zendedel A, Khodadadi A, Mohammadi-asl J. Does prepubertal testicular tissue vitrification influence spermatogonial stem cells (SSCs) viability? *J Assist Reprod Genet* 2013; 30: 1271-1277.
35. Forouzanfar M, Abid A, Hosseini SM, Hajian M, Nasr Esfahani MH. Supplementation of sperm cryopreservation media with cell permeable superoxide dismutase mimetic agent (MnTE) improves goat blastocyst formation. *Cryobiology* 2013; 67: 394-397.
36. Forouzanfar M, Fekri Ershad S, Hosseini SM, Hajian M, Ostad-Hosseini S, Abid A, et al. Can permeable super oxide dismutase mimetic agents improve the quality of frozen-thawed ram semen? *Cryobiology* 2013; 66: 126-130.
37. Tan DX, Manchester LC, Liu X, Rosales-Corral SA, Acuna-Castroviejo D, Reiter RJ. Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. *J Pineal Res* 2013; 54: 127-138.
38. Gholami M, Saki G, Hemadi M, Khodadadi A, Mohammadi-asl J. Melatonin improves spermatogonial stem cells transplantation efficiency in azoospermic mice. *Iran J Basic Med Sci* 2014; 17: 93.
39. Izzo G, Ferrara D, Napolitano F, Crispo AA, d'Istria M, Aniello F, et al. Identification of a cDNA encoding for Ghrelin in the testis of the frog Pelophylax esculentus and its involvement in spermatogenesis. *Comparative Biochem PhysiolPart A: Mol Integr Physiol* 2011; 158: 367-373.
40. Egecioglu E, Prieto-Garcia L, Studer E, Westberg L, Jerlhag E. The role of ghrelin signalling for sexual behaviour in male mice. *Addiction Biol* 2016; 21: 348-359.
41. Sastry MK, Mohammed C. The summary-comparison matrix: A tool for writing the literature review. IEEE International Professonal Communication 2013 Conference, Canada; 2013.
42. Kushki D, Azarnia M, Gholami M. Antioxidant Effects of Selenium on Seminiferous Tubules of Immature Mice Testis. *Zahedan J Res Med Sci* 2015; 17: 29-33.
43. Khaki A, Fathiazad F, Nouri M, Khaki A, Maleki NA, Khamnei HJ, et al. Beneficial effects of quercetin on sperm parameters in streptozotocin- induced diabetic male rats. *Phytother Res* 2010; 24: 1285-1291.
44. Khaki AA, Khaki A, Nouri M, Ahmadi-Ashtiani HR, Rastegar H, Rezazadeh S, et al. Evaluation effects of Quercetin on liver apoptosis in streptozotocin-induced diabetic rat. *J Med Plants* 2009; 8 (Suppl.): 70-78.
45. Khaki A, Nouri M, Fathiazad F, Ahmadi-Ashtiani HR, Rastgar H, Rezazadeh S. Protective effects of Quercetin on spermatogenesis in streptozotocin-induced diabetic rat. *J Med Plants* 2009; 8 (Suppl.): 57-64.
46. Khaki A, Ghanbari Z, Ghanbari M, Ouladsahebmadarek E, Javadi L, Farzadi L, et al. Anti-oxidative effects of citro flavonoids on spermatogenesis in rat. *Afr J Pharm Pharmacol* 2011; 5: 721-725.
47. Khaki A, Fathiazad F, Nouri M, Khaki AA. Effect of Ocimum basilicum on apoptosis in testis of rats after exposure to electromagnetic field. *Afr J Pharm Pharmacol* 2011; 5: 1534-1537.
48. Asghari A, Akbari G, Galustanian G. Magnesium Sulfate Improves Sperm Characteristics Against Varicocele in Rat. *Crescent J Med Biol Sci* 2016; 3: 55-59.
49. Ouladsahebmadarek E, Giasi GS, Khaki A, Ahmadi Y, Farzadi L, Ghasemzadeh A, et al. The effect of

compound herbal remedy used in male infertility on spermatogenesis and pregnancy rate. *Int J Women's Health Reprod Sci* 2016; 4: 195-198.
50. Khaki A, Khaki A. Effect of cinnamomum zeylanicumon on spermatogenesis. *Iran Red Crescent Med J* 2015; 17: e18668.
51. Zahedi A, Fathiazad F, Khaki A, Ahmadnejad B. Protective effect of ginger on gentamicin-induced apoptosis in testis of rats. *Adv Pharm Bulletin* 2012; 2: 197-200.
52. Khaki A, Farnam A, Rouhani S, Imantalab B, Seery S. Androgenic activity evaluation of ginger rhizome in reducing depression in the forced swimming test of rats Exposed to Electromagnetic Field (EMF). *Int J Women's Health Reprod Sci* 2013; 1: 56-63.
53. Hoseinpouran M, Khaki A, Nazem H. Assessment of Antioxidant Properties of *Allium cepa* on Serum Antioxidants and Spermatogenesis After Consuming Tartrazine in Rat. *Crescent J Med Biol Sci* 2015; 2: 125-129.
54. Alizadeh H, Khaki A, Farzadi L, Nouri M, Ahmadi-Asrbadr Y, Seyed-Ghiasi G, et al. The Therapeutic Effects of a Medicinal Plant Mixture inCapsule Form on Catalase Levels in the Semen of Men withOligospermia. *Crescent J Med Biol Sci* 2015; 2: 6-9.
55. Khaki A, Fathiazad F, Nouri M, Khaki AA, Ozanci CC, Ghafari-Novin M, et al. The effects of Ginger on spermatogenesis and sperm parameters of rat. *Iran J Reprod Med* 2009; 7: 7-12.
56. Nouri M, Khaki A, Azar FF, Rashidi MR. The protective effects of carrot seed extract on spermatogenesis and cauda epididymal sperm reserves in gentamicin treated rats. *Yakhteh* 2009; 11: 327-33.
57. Asghari A, Akbari G, Beigi AM, Mortazavi P. Effects of Tramadol Administration on Sperm Characteristics on Testicular Ischemia-Reperfusion Injury in Rat. *Crescent J Med Biol Sci* 2016; 3: 119-122.
58. Kushki D, Azarnia M, Khanipour-Khayat Z, Beigi-Boroujeni M, Moradian-Majd A, Gholami M. Effects of Vitamins E and C on Freezed-Thawed Immature Mice Testis. *Zahedan J Res Med Sci* 2016; (In Press).
59. Mitra A, Mandana B. Application of gel-based proteomic technique in female reproductive investigations. *J Hum Reprod Sci* 2015; 8: 18-24.
60. Badr F, El Habit O, Harraz M. Radioprotective effect of melatonin assessed by measuring chromosomal damage in mitotic and meiotic cells. *Mutat Res Genet Toxicol Environment Mutagen* 1999; 444: 367-372.
61. Deng SL, Chen SR, Wang ZP, Zhang Y, Tang JX, Li J, et al. Melatonin promotes development of haploid germ cells from early developing spermatogenic cells of Suffolk sheep under in vitro condition. *J Pineal Res* 2016; 60: 435-447.
62. Gholami M, Saki G, Hemadi M, Khodadadi A. Effect of melatonin on the expression of apoptotic genes in vitrified-thawed spermatogonia stem cells type A of 6-day-old mice. *Iran J Basic Med Sci* 2013; 16: 906-909.
63. Hajiaghalou S, Ebrahimi B, Shahverdi A, Sharbatoghli M, Beigi Boroujeni N. Comparison of apoptosis pathway following the use of two protocols for vitrification of immature mouse testicular tissue. *Theriogenology* 2016; 86: 2073-2082.
64. Boroujeni MB, Salehnia M, Khalatbary AR, Pourbeiranvand S, Boroujeni NB, Ebrahimi S. Effect of ovarian stimulation on the endometrial apoptosis at implantation period. *Iran Biomed J* 2010; 14: 171-177.
65. Ahmadi SAY, Shahsavar F, Akbari S. A Review on Controversies about the Role of Immune and Inflammatory Systems in Implantation Process and Durability of Pregnancy. *Int J Women's Health Reprod Sci* 2016; 4: 96-102.
66. Yasin Ahmadi SA, Tavafi M, Ahmadi PS. A critical approach to administration of low-dose aspirin (LDA) to improve implantation success. *Int J Women's Health Reprod Sci* 2015; 3: 223-224.
67. Ahmadvand H, Ahmadi SAY, Sayahi A, Rezaian J. Role of Apoptosis in CNS Emphasizing Spinal Cord Injuries: A Commentary. *Iran J Neurosurg* 2016; 1: 30-31.
68. Ghasemi FM, Faghani M, Khajehjahromi S, Bahadori M, Nasiri E, Hemadi M. Effect of melatonin on proliferative activity and apoptosis in spermatogenic cells in mouse under chemotherapy. *J Reprod Contracept* 2010; 21: 79-94.
69. Gholami M, Saki G, Hemadi M, Khodadadi A, Mohammadiasl J. Melatonin Effect on Immature Mouse Testicular Tissues, Vitrified-Thawed With Different Cryoprotectant Media*. Jentashapir J Health Res* 2015; 6: .
70. Yang W-C, Tang K-Q, Fu C-Z, Riaz H, Zhang Q, Zan L-S. Melatonin regulates the development and function of bovine Sertoli cells via its receptors MT1 and MT2. Anim Reprod Sci 2014; 147: 10-16.
71. d'Istria M, Palmiero C, Serino I, Izzo G, Minucci S. Inhibition of the basal and oestradiol-stimulated mitotic activity of primary spermatogonia by melatonin in the testis of the frog, Rana esculenta, in vivo and in vitro. *Reproduction* 2003; 126: 83-90.
72. Saki G, Mirhoseini M, Hemadi M, Khodadadi A, Amiri FBT. The effect of the melatonin on cryopreserved mouse testicular cells. *Int J Reprod BioMed* 2016; 14: 23.
73. Hemadi M, Abolhassani F, Akbari M, Sobhani A, Pasbakhsh P, Ährlund-Richter L, et al. Melatonin promotes the cumulus-oocyte complexes quality of vitrified-thawed murine ovaries; with increased mean number of follicles survival and ovary size following heterotopic transplantation. *Eur J Pharmacol* 2009; 618: 84-90.
74. Hemadi M, Zargar M, Sobhani A, Sobhani A. Assessment of morphological and functional changes in neonate vitrified testis grafts after host treatment with melatonin. *Folia Morphol (Warsz)* 2011; 70: 95-102.
75. Tena-Sempere M. Exploring the role of ghrelin as novel regulator of gonadal function. *Growth Hormone and IGF Res* 2005; 15: 83-88.
76. Olejniczak K, Ruciński M, Rucha a M, Sowiński J. Immunohistochemical and hybridocytochemical study on ghrelin signalling in the rat seminiferous

epithelium. *Folia Histochemica et Cytobiologica* 2009; 47: 415-423.
77. Whirledge SD, Garcia JM, Smith RG, Lamb DJ. Ghrelin partially protects against cisplatin-induced male murine gonadal toxicity in a GHSR-1a-dependent manner. *Biol Reprod* 2015; 92: 76.
78. Garcia JM, Chen J-a, Guillory B, Donehower LA, Smith RG, Lamb DJ. Ghrelin prevents cisplatin-induced testicular damage by facilitating repair of DNA double strand breaks through activation of p53. *Biol Reprod* 2015;115: 129759.