Journal of Current Biomedical Reports
Volume 4, Number 1, 2023
jcbior.com
eISSN: 2717-1906

Original research

Histopathological changes of spermatogenesis induced by Intra-peritoneal injection of CuO nanoparticles

Ghazaleh Larijani[1,2], Mahsa Motamed[3], Arezoo Maleki-Hajiagha[3], Kazem Parivar[1], Nasim Hayati Roodbari[1], Sima Vaez[1,*], Naser Amini[2]

[1]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Cellular and Molecular Research Centre, Iran University of Medical Sciences, Tehran, Iran*
[3]*Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

Abstract

A number of lifestyle-related factors, including smoking and obesity, as well as a number of environmental factors, such as exposure to dioxins, combustion products, and traffic exhaust gases, appear to have a deleterious influence on the testes and spermatogenesis. Because of its oxidation-reduction (Redox) potential, copper functions as a cofactor in a variety of enzymes that are essential for basic metabolic functions. We assess how copper oxide nanoparticles (CuO-NPs) affected the male reproductive system and the probability of getting pregnant, both of which have not yet been studied. Four groups of creatures were chosen at random. The experimental group received daily doses of 50, 100, and 150 mg/kg of CuO-NPs intraperitoneally (IP). According to the results of the histological investigation, CuO NPs treatment significantly enhanced Sertoli and Leydig cells as well as all testicular germ cells other than spermatozoa at a dose of 100 mg/kg in both the in vivo and in vitro stages. The 150 mg group's spermatids and spermatocytes had significantly decreased as a result of CuO NP's harmful effects during the in vitro phase ($P < 0.05$). We can therefore conclude that a buildup of CuO in high doses may result in a disruption of the CuO homeostasis, which may subsequently reduce male fertility.

Keywords: Cellular biology, CuO, Nanoparticle, Spermatogenesis

1. Introduction

Seminiferous tubules (STs) are the site of the intricate process known as spermatogenesis, which results in the development of the mature male gamete [1]. Recent research suggests that the entire spermatogenic process in healthy males takes between 42 and 76 days [2] and involves a variety of cells, hormones, paracrine agents, genes, and epigenetic variables [1]. Numerous factors have been shown to affect sperm quality and quantity. Testes and spermatogenesis appear to be negatively impacted by a number of lifestyle-related factors, including obesity and smoking, as well as a number of environmental factors, including exposure to dioxins, combustion products, and traffic exhaust fumes [3]. Many studies concentrate on specific minerals and biomaterials to aid in reducing harmful effects and enhancing the effectiveness of spermatogenesis [4-6].

Copper (Cu) is a necessary element for the proliferation and normal development of all living organisms [7]. Copper is a cofactor in numerous enzymes responsible for fundamental metabolic

[*]**Corresponding author:**
Sima Vaez, Ph.D
Department of Biology, Science and Research Branch,
Islamic Azad University, Tehran, Iran
Tel/Fax: +98 21 44845205
Email: vaezmrs@gmail.com
http://orcid.org/0009-0001-0308-5228

Received: September, 20, 2022
Accepted: December, 20, 2022

processes because of its oxidation-reduction (Redox) potential [8]. Because both its decline and excessive rise can result in problems with male reproductive function, microelements such as copper plays a unique and significant role in how affect male fertility [7]. High concentrations of copper have been linked to decreased spermatozoa motility and number, as well as seminiferous tubule epithelial deterioration [9-11]. On the other side, there is strong evidence that indicates copper deficiency results in reduced spermatozoa quantity and motility as well as lower ejaculate volume [12, 13]. These studies all indicated that the concentration of copper in the testes should be carefully regulated.

Nanoparticles (NPs) are matter particles with a size range of 1 to 100 nanometers that are commonly categorized as nanoscale in terms of their mobility and physical features (nm). Due to their extremely small size and huge surface area, NPs are widely used in a variety of fields. [14]. It is necessary to measure the effects of this nanoparticle in biological ways, especially on the reproductive system, due to the widespread demand and application of CuO-NPs in a variety of industries, including water treatment, textile manufacturing, food preservation, and agricultural practices [15], But the body of knowledge on this crucial topic is still lacking. As a result, in a recent study, we assessed the effects of CuO-NPs on the male reproductive system and the potential for pregnancy, both of which have not yet been investigated.

2. Materials and Methods

2. 1 Experimental animals

Thirty healthy adult male NMRI (Naval Medical Research Institute) mice (6–8 weeks old) weighing 28–32g were used in this study (Pasteur Institute, Tehran, Iran). The ethics committee of The Islamic Azad University's Science and Research Branch in Tehran gave its approval to this work. The animals were kept in typical laboratory conditions for at least a week before the experiment (i.e., 12 hours of darkness followed by 12 hours of light, 50% relative humidity, and 22 °C). Commercial rodent food (pellet) and water were available, and animal cages were kept free.

2.2 Experimental design

The mice were divided into five groups (one control, one sham and three experimental groups) for the in vivo studies and five groups (control, sham and three experimental groups) for the in vitro experiments. Each group had three mice. Both adult mice's testicles were taken out and cultured for two days with CuO-NPs as a supplement for the in vitro experimental groups. After that, fix and gauge. Drug solvent was used as a supplement for the sham group. For the in vivo groups, each experimental group received various dosages of CuO-NPs intraperitoneally via a single daily injection for five days, including 50 mg/kg, 100 ml/kg, and 150 mg/kg. As with the other groups, the fictitious (sham) group received 1ml of distilled water. The intervention-free group served as the control. All groups were sacrificed under ether anesthesia eighteen days following the initial injection, and the testicles from each animal were dissected with Surgical Blade and placed in a Petri dish under sterile conditions.

2.3 Preparation of CuO-NPs solution

The distilled water was combined with the powdered copper oxide nanoparticles and vortexed. The solution was promptly injected into the animals in various doses based on the group to avoid powder settling.

2.4 Sample preparation for histopathological analysis

After surgery of mice and isolation of testes, each mouse's testes were embedded in paraffin after being dried in a graduated series of ethanol and fixed in Bouin's solution. Hematoxylin and eosin (H&E) staining was done on the samples after they had been sectioned by a microtome into 6 mm thick sections for histopathological analysis under a light microscope.

2.5 The weight evaluation of mice in different groups

Adult mice had their testes removed, and measurements were made of their weights, testicular sizes, and testes tubule and epididymis tubule diameters with ruler and scale.

2.6 Histopathological analysis

In several mouse groups, the quantity of Sertoli cells, Leydig cells, fibroblasts, blood vessels, seminiferous tubules, and epididymis tubules were counted. Various groups of mature sperms, spermatocytes, and spermatids were assessed in addition to the quantity of various spermatogonial

stem cells. Each sample was averaged after being counted four times.

2.7 Statistical analysis

The study's data were examined using SPSS software, version 21. Analysis of variance was used to compute and examine the statistical significance across groups using the one-way ANOVA test. At P 0.05, the differences were deemed significant.

3. Results

3.1 The weight changes of mice

The weight of mice treated with CuO-NPs does not significantly change after intraperitoneal injection. The mice's minor weight rise is caused by the right food storage and use conditions, although this change was not significantly different from the control group (Supplementary Table 1).

3.2 The histopathological effects of CuO-NPs on the testes of mice

Adult mice had their testes removed, and they were weighed. The findings of the macro and microscopic examination revealed that CuO-NPs had no appreciable impact on testicular weights, testicular dimensions, testicular tubule diameter, or epididymis tubules (Supplementary Table 2).

3.3 The histopathological effects of CuO-NPs on the testicular cells of mice

According to our findings, mice who received CuO-NPs at a dose of 100 mg/kg had significantly more Sertoli cells, Leydig cells, fibroblasts, seminiferous tubules, and epididymis tubules. Blood vessels, however, showed no discernible changes. We did not observe any statistically significant difference in the 50 mg/kg group. Sertoli cells, blood vessels, and Seminiferous tubules were all less prevalent in the 150 mg/kg group, although Leydig cells and epididymis tubules were more prevalent. Only the decrease in Sertoli cells among the aforementioned alterations in the 150 mg/kg group was statistically significant (Supplementary Table 3).

3.4 The histopathological effects of CuO-NPs on the testicular germ cells of mice

Our findings demonstrated that animals given CuO-NPs at a dose of 100 mg/kg had an increase in the number of germ cells at all developmental stages, including B-type spermatogonia, spermatocytes, spermatids, and epididymal sperm. With the exception of epididymal sperms, this increase was statistically significant in all developmental stages. Additionally, although the difference between the control group and animals administered CuO-NPs at doses of 50 and 150 mg/kg was not statistically significant, the mice received somewhat more germ cells (Supplementary Table 4).

In the in vivo phase of the investigation, mice that received CuO-NPs at a dose of 100 mg/kg had a rise in the number of germ cells in all developmental stages, such as B-type spermatogonia, spermatocytes, spermatids, as well as the number of Sertoli cells and fibroblasts. All types of germ cells had this rise, which was statistically significant. However, Sertoli and fibroblastic cells did not experience it. Despite the in vivo phase results, the number of type B spermatogonial stem cells, spermatocytes, and spermatids dropped in the 150 m/kg group. For spermatocytes and spermatids, this decrease was statistically significant. Although Sertoli and fibroblastic cell counts fell and the total number of germ cells increased somewhat in the 50 m/kg group, none of these changes were statistically significant.

In this study, we indicated there were no significant differences between the 50 and 150 mg/kg groups (37±1.3 and 32±2.5, respectively), while the number of type B spermatogonial stem cells was considerably larger in the 100 mg/kg group than the control group (Figure 1). Additionally, the 100 mg/kg group had significantly more spermatocytes than the other groups (66.8±1.4), whereas the 150 mg/kg group had the fewest (21±2.3). These modifications both had statistical significance. The 50 mg/kg group did not differ significantly from the control group in any way (Figure 2). The number of spermatids was found to be equivalent to our spermatocyte number findings. The number of spermatocytes was significantly larger in the 100 mg/kg group than in any other group (268±5.5), whilst the 150 mg/kg group had the fewest (101±4.4). These modifications both had statistical significance. The 50 mg/kg group did not differ significantly from the control group in any way (Figure 3). Figures 4 and 5, respectively, depict alterations in fibroblasts and Sertoli cells. Between the groups, no discernible differences in these two types of cells were seen. Histopathological evaluation of

spermatogenesis cells showed in Figure 6 using H&E staining.

4. Discussion

Due to its potential for usage in pharmacological and biomedical applications, copper nanoparticles are widely used in a variety of industries. Consumption of copper nanoparticles above the allowable limit manifests harmful effects that result in cytotoxicity and cell death. Despite having differing outcomes in the in vivo and in vitro study phases, we were able to demonstrate that CuO-NPs have dose-dependent effects on the male mice's reproductive system in the current study. At a dose of 100 mg/kg in both the in vivo and in vitro phases, CuO-NPs treatment significantly increased Sertoli and Leydig cells as well as all testicular germ cells other than spermatozoa in epididymal tables. While in the in vitro phase, it was linked to a significant decrease in spermatids and spermatocytes and a non-significant decrease in spermatogonial stem cells, at the higher dose of 150 mg/kg, it caused a non-significant increase of all germ cells and decrease of spermatozoa in epididymal tubules in the *in vivo* study. Numerous theories exist regarding the function of Cu in reproduction, and the findings of earlier investigations are quite erratic. Cu has been identified as a significant trace element that is particularly vital for the male reproductive system and has a critical ecophysiological role in a variety of cells and tissues. Therefore, it is essential for healthy reproduction and good spermatogenesis to consume this vitamin on a daily basis in a fair amount. Ceruloplasmin, superoxide dismutase (SOD1, SOD3, and Cu/Zn SOD) group of metallothionein, and cytochrome c oxidase (COX), which are all present at all stages of gametogenesis as well as in the somatic cells of the testis and epididymis, are just a few examples of the bioactive molecules and enzymes that require copper as an essential metal cofactor [12, 16]. Due to Cu's redox potential, one of its crucial functions in the male reproductive system is participation in the balancing of oxidative stress status and protecting cellular structure and function from oxidative damage [17]. This is because Cu is one of the key components of the SOD [18], a key antioxidant enzyme that dismutases O2 to produce oxygen (O2) and H2O2. Diamine oxidase (DAO), another significant Cu-dependent enzyme, is present in very high quantities in seminal human plasma and is responsible for the breakdown of polyamines [12, 16, 19]. Additionally, it has been demonstrated that azoospermic males have lower levels of Cu in their seminal plasma than healthy men [20, 21]. Cu significantly improved sperm count, sperm motility, semen volume, and progressive motility [22, 23].

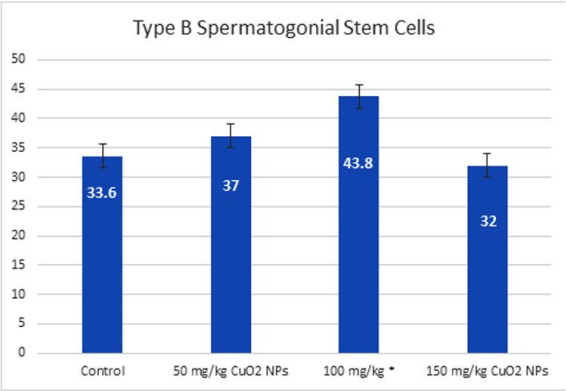

Figure 1. Comparing the number of Spermatogonial stem cells in different groups of mice at *in vitro* phase of study (Data is presented as mean and standard deviation [SD]; *P <0.05).

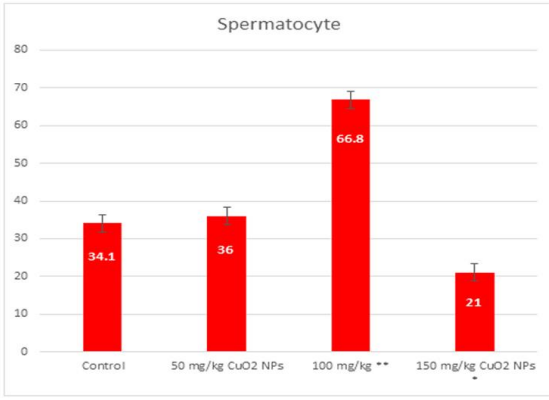

Figure 2. Comparing the number of Spermatocytes in different groups of mice at *in vitro* phase of study (Data is presented as mean and standard deviation [SD]; *P <0.05; **P <0.01).

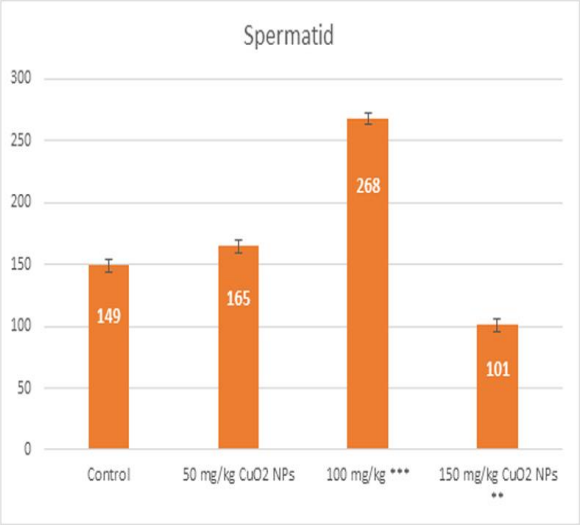

Figure 3. Comparing the number of Spermatids in different groups of mice at *in vitro* phase of study (Data is presented as mean and standard deviation [SD]; ** P<0.01).

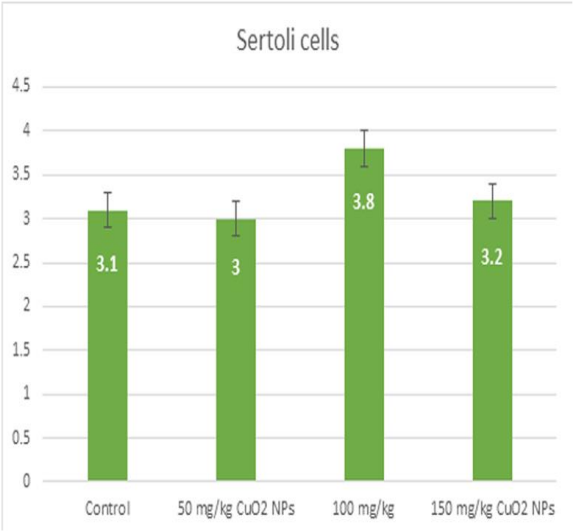

Figure 4. Comparing the number of Sertoli cells in different groups of mice at *in vitro* phase of study (Data is presented as mean and standard deviation).

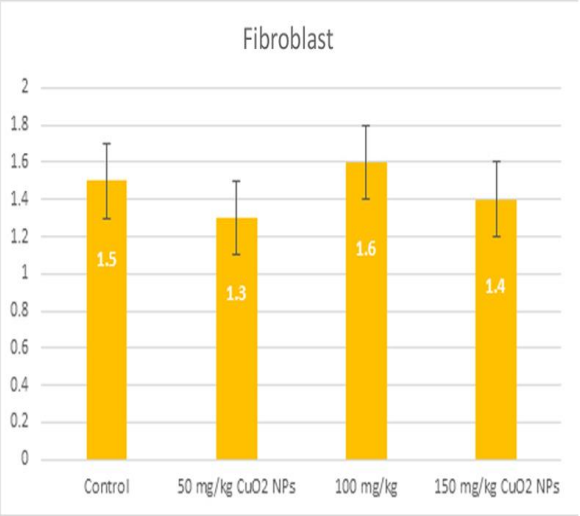

Figure 5. Comparing the number of fibroblasts in different groups of mice at *in vitro* phase of study (Data is presented as mean and standard deviation).

According to the findings of an in vitro study using buffalo ejaculate, copper addition to semen culture media at low doses may increase the total antioxidant capacity and provide better protection of sperms through the processes of dilution, equilibration, and freeze-thawing than that in control. However, higher concentrations had detrimental effects on the sperm [24]. Another study had shown that the enzymatic activity of Cu/Zn SOD Cu, which defends spermatogonial stem cells during early spermatogenesis against ROS attack, was boosted when Cu was added to the testicular cell culture [25]. Because Celino and Tabassomi's financing supported the positive effect of copper on sperm quality, the findings of their study were identical to those of Anchordoquy et al. they demonstrated that adding copper sulfate at a concentration of 40 g/dL to the medium used to cultivate bovine spermatozoa

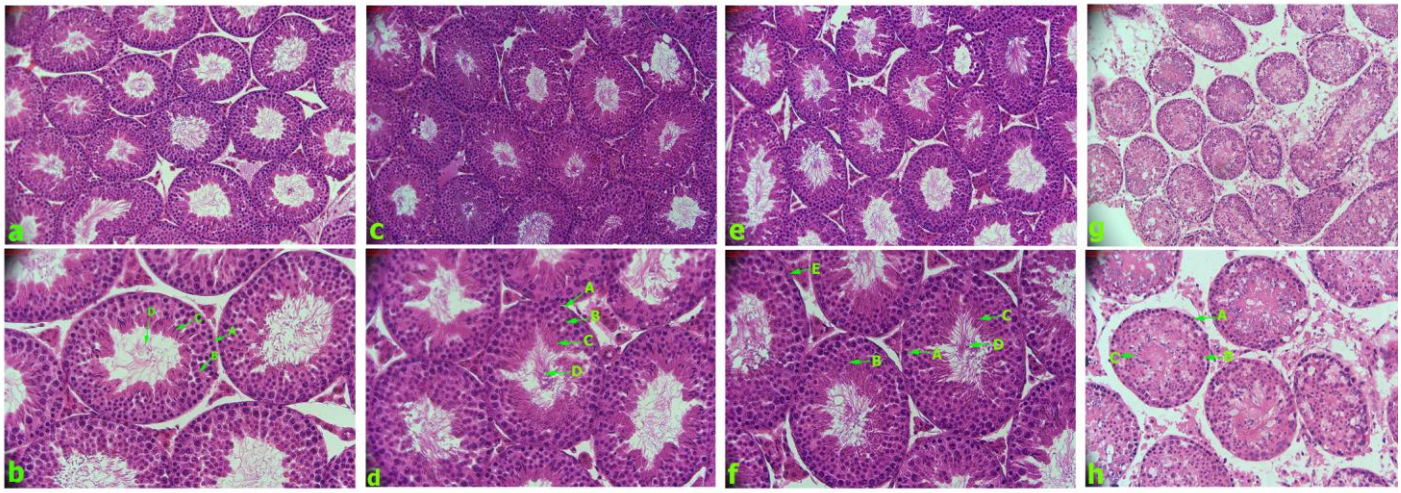

Figure 6: Histopathological evaluation of spermatogenesis using H&E staining: a; control group with10x magnification, b: control group with 20x magnification, c; dose 50 mg/kg group with10x magnification, d: dose 50 mg/kg group with 20x magnification, e; dose 100 mg/kg with10x magnification, d: dose 100 mg/kg with 20x magnification, g; dose 150 mg/kg with10x magnification, h: dose 150 mg/kg with 20x magnification. In section b, d, f, and h: A; Spermatogonia B; Spermatocyte C; spermatid D; Sperm E. Sertoli.

improved total and progressive sperm motility, sperm viability, and functional sperm membrane integrity [26]. Free Cu is a highly reactive element that has the potential to produce free radicals, which can seriously damage proteins and DNA [27]. Copper oxide nanoparticles may prevent the release of gonadotropins and testosterone, which would ultimately prevent the spermatogenesis process from taking place [28]. Rajabi et al. research are also revealed that exposure to CuO-NPs disrupts the process and structure of gonadal spermatogenesis and causes oxidative stress and cellular disintegration [15]. Male rats' blood-testis barriers have been shown to be permeable by CU NPs, which can then kill Sertoli, Leydig, and germ cells [29]. Additionally, oral Cu treatment in rats causes testicular structural abnormalities such as atrophic and sclerotic tubules and an increase in apoptosis, along with a decrease in spermatogonial and Sertoli cells [8]. Guo et al. have reported the most likely molecular mechanism of the disordered spermatogenesis caused by copper sulfate ($CuSO_4$) [31]. They demonstrated how $CuSO_4$ decreases antioxidant activity while increasing ROS generation. Additionally, $CuSO_4$ increased the amounts of cleaved caspase-3, cleaved caspase-8, cleaved caspase-9, caspase-12, cleaved-PARP, and Bax while simultaneously decreasing the expression of Bcl-2. Eventually, sperm concentration and motility are decreased, sperm concentration is decreased, and the rate of sperm malformation is increased due to oxidative stress-mediated DNA damage and germ cell apoptosis. Additionally, it has been demonstrated in another study that exposure to $CuSO_4$ in rats lowers the quality of their sperm, inhibits the release of the sex hormone and gonadotropin, as well as the anti-oxidative activity of the testes, which in turn reduces spermatogenesis [11]. Recently, Chen et al. also looked into the causes and molecular mechanisms of CuO-NPs cytotoxicity. They discovered that sperm quality, fructose content, and sex hormone release was reduced after 28 days of exposure to CuO-NPs at doses of 44, 88, and 175 mg/kg/day. Additionally, nano-copper enhanced the rate of sperm malformation and the amount of oxidative stress and caused aberrant structural alterations in testicular tissue. They came to the conclusion that cell apoptosis and autophagy are closely associated to nano-copper-induced damage after excessive exposure in testicular tissues and spermatogenesis through controlling the Akt/mTOR signaling pathway [32].

CuO-NPs was introduced directly to the culture media in free form during in-vitro culture, which as previously noted can result in significant oxidative damage [33]. We believe that the increased number of somatic and spermatogenic cells in the testis at a dose of 100 mg/kg may be related to copper's crucial role in

maintaining the balance of oxidative stress as well as its involvement in the processes of both mitotic and meiotic cell divisions, as it may activate mitosis cycles and cell divisions and speed up growth and development processes at physiological or slightly elevated concentrations. However, with increasing doses, this relationship may become more pronounced. We discovered through histological analysis that the epididymis had thicker tunica and tubes. These results support the hypothesis that CuO-NPs exposure at large concentrations inhibits cell development and proliferation and, combined with its toxic effects, causes cell and tissue necrosis and degeneration. Additionally, as the dose was increased, spermatids gathered in the lumen of the seminiferous tubules, indicating that differentiation had stopped. In addition, we observed that the number of spermatogenic cells dropped, the tunica's thickness rose, and the tubules developed pits in histological analyses. There were many atrophic cells in the in vitro samples, and the treated sample had more degenerated cells than the control, which supports the harmful effects of high-dose CuO-NPs. Overall, the findings of this study demonstrate that CuO-NPs has a dual potential that includes both toxicity and the promotion of growth and development processes. This chemical is highly hazardous at large concentrations and can cause tissue necrosis as well as cell death.

Due to this study, we can draw the conclusion that an accumulation of Cu in high dosages may cause a disruption of the Cu homeostasis, which might therefore impair male fertility. It follows that a considerable decrease in male fertility, anomalies at the sperm level, male gonad, and sex hormone synthesis are caused by both copper excess and deficiency. Consequently, we should refer to copper as "a double-edged sword" in the context of male fertility. Also we reasoned that the differences in outcomes between the *in vitro* and *in vivo* phases might be due to the blood-testis barrier present in living animals, which would delay the rate of CuO-NPs exposure in comparison to direct exposure in the *in vitro*.

Authors' contributions

Conceptualization, original draft: GL, SV; Draft Preparation, writing, review, editing: MM, AM, NA; Supervision and project administration, draft Preparation: KP, NH. All authors read and approved the final version of manuscript.

Conflict of interests

None.

Ethical declarations

All procedures were performed in accordance with the guidelines of the Medical Ethics Committee of Islamic Azad University Science and Research Branch with register number 910808.

Financial support

This research work was funded by The Islamic Azad University Science and Research Branch.

Supplementary files

Supplementary file 1.

References

1. Neto FTL, Bach PV, Najari BB, Li PS, Goldstein M, editors. Spermatogenesis in humans and its affecting factors. Semin Cell Dev Biol. 2016; 59:10-26.
2. Misell L, Holochwost D, Boban D, Santi N, Shefi S, Hellerstein M, Turek P. A stable isotope-mass spectrometric method for measuring human spermatogenesis kinetics in vivo. J Urol. 2006; 175(1):242-6.
3. Sharpe RM. Environmental/lifestyle effects on spermatogenesis. Philos Trans R Soc Lond B Biol Sci. 2010 ; 365(1546):1697-712.
4. Talebi AR, Khorsandi L, Moridian M. The effect of zinc oxide nanoparticles on mouse spermatogenesis. J Assist Reprod Genet. 2013; 30(9):1203-9.
5. de Lourdes Pereira M, Costa FG. Spermatogenesis recovery in the mouse after iron injury. Hum Exp Toxicol. 2003; 22(5):275-9.
6. Lin S, Qiao N, Chen H, Tang Z, Han Q, Mehmood K, et al. Integration of transcriptomic and metabolomic data reveals metabolic pathway alteration in mouse spermatogonia with the effect of copper exposure. Chemosphere. 2020; 256:126974.
7. Herman S, Lipiński P, Ogórek M, Starzyński R, Grzmil P, Bednarz A, Lenartowicz M. Molecular regulation of copper homeostasis in the male gonad during the process of spermatogenesis. Int J Mol Sci. 2020; 21(23):9053.
8. Ogórek M, Gąsior Ł, Pierzchała O, Daszkiewicz R, Lenartowicz M. Role of copper in the process of spermatogenesis. Postepy Hig Med Dosw (Online). 2017; 71(0):663-683.
9. Kowal M, Lenartowicz M, Pecio A, Gołas A, Błaszkiewicz T, Styrna J. Copper metabolism disorders affect testes structure and gamete quality in male mice. Syst Biol Reprod Med. 2010; 56(6):431-44.
10. Kasperczyk A, Dobrakowski M, Czuba ZP, Kapka-Skrzypczak L, Kasperczyk S. Environmental exposure to zinc and copper influences sperm quality in fertile males. Ann Agric Environ Med. 2016; 23(1):138-43.
11. Liu J, Yang X, Sun X, Zhuang C, Xu F, Li Y. Suppressive effects of copper sulfate accumulation on the spermatogenesis of rats. Biol Trace Elem Res. 2016; 174(2):356-61.

12. Tvrda E, Peer R, Sikka SC, Agarwal A. Iron and copper in male reproduction: a double-edged sword. J Assist Reprod Genet. 2015; 32(1):3-16.
13. Aupperle H, Schoon H, Frank A. Experimental copper deficiency, chromium deficiency and additional molybdenum supplementation in goats–pathological findings. Acta Vet Scand. 2001; 42(3):311-21.
14. Hickey JW, Santos JL, Williford J-M, Mao H-Q. Control of polymeric nanoparticle size to improve therapeutic delivery. J Control Release. 2015; 219:536-47.
15. Ighalo JO, Sagboye PA, Umenweke G, Ajala OJ, Omoarukhe FO, Adeyanju CA, et al. CuO nanoparticles (CuO NPs) for water treatment: A review of recent advances. Environ Nanotechnol Monit Manag. 2021; 15:100443.
16. Arredondo M, Núñez MT. Iron and copper metabolism. Mol Aspects Med. 2005; 26(4-5):313-27.
17. Uriu-Adams JY, Keen CL. Copper, oxidative stress, and human health. Mol Aspects Med. 2005; 26(4-5):268-98.
18. Peeker R, Abramsson L, Marklund SL. Superoxide dismutase isoenzymes in human seminal plasma and spermatozoa. Mol Hum Reprod. 1997; 3(12):1061-6.
19. Calvé ML, Segalen J, Quernee D, Lavault M, Lescoat D. Andrology: Diamine oxidase activity and biochemical markers in human seminal plasma. Hum Reprod. 1995; 10(5):1141-4.
20. Saleh BOM, Hussain NK, Majid AY, Thabet B, Fadhil KA. Status of zinc and copper concentrations in seminal plasma of male infertility and their correlations with various sperm parameters. Iraq Postgrad Med J. 2008; 7(1):76-80.
21. Akinloye O, Abbiyesuku FM, Oguntibeju OO, Arowojolu AO, Truter EJ. The impact of blood and seminal plasma zinc and copper concentrations on spermogram and hormonal changes in infertile Nigerian men. Reprod Biol. 2011; 11(2):83-97.
22. Machal L, Chladek G, Strakova E. Copper, phosphorus and calcium in bovine blood and seminal plasma in relation to semen quality. J Anim Plant Sci. 2002; 11(3):425-36.
23. Wong WY, Flik G, Groenen PM, Swinkels DW, Thomas CM, Copius-Peereboom JH, et al. The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. Reprod Toxicol. 2001; 15(2):131-6.
24. Tabassomi M, Alavi-Shoushtari SM. Effects of in vitro copper sulphate supplementation on the ejaculated sperm characteristics in water buffaloes (Bubalus bubalis). Vet Res Forum. 2013; 4(1):31-6.
25. Celino FT, Yamaguchi S, Miura C, Ohta T, Tozawa Y, Iwai T, Miura T. Tolerance of Spermatogonia to Oxidative Stress Is Due to High Levels of Zn and Cu/Zn Superoxide Dismutase. Plos one. 2011; 6(2):e16938.
26. Anchordoquy JP, Anchordoquy JM, Pascua AM, Nikoloff N, Peral-García P, Furnus CC. The copper transporter (SLC31A1/CTR1) is expressed in bovine spermatozoa and oocytes: Copper in IVF medium improves sperm quality. Theriogenology. 2017; 97:124-33.
27. Valko M, Jomova K, Rhodes CJ, Kuča K, Musílek K. Redox-and non-redox-metal-induced formation of free radicals and their role in human disease. Arch Toxicol. 2016; 90(1):1-37.
28. Rajabi S, Noori A, Shahbazi F. Copper oxide nanoparticles toxicity on pituitary gonadal axis and spermatogenesis in male rats. J Gorgan Univ Med Sci. 2019; 21(3):49-56.
29. Rajabi S, Shahbazi F, Noori A, Karshenas R. Investigation of the toxic effects of copper oxide nanoparticles on the structure of gonadal tissue in male. Exp Anim Biol. 2019; 7(4):117-24.
30. Babaei H, Kheirandish R, Ebrahimi L. The effects of copper toxicity on histopathological and morphometrical changes of the rat testes. Asian Pac J Trop Biomed. 2012; 2(3):S1615-S9.
31. Guo H, Ouyang Y, Wang J, Cui H, Deng H, Zhong X, et al. Cu-induced spermatogenesis disease is related to oxidative stress-mediated germ cell apoptosis and DNA damage. J Hazard Mater. 2021; 416:125903.
32. Chen H, Wang Y, Luo J, Kang M, Hou J, Tang R, et al. Autophagy and apoptosis mediated nano-copper-induced testicular damage. Ecotoxicol Environ Saf. 2022; 229:113039.
33. Lovejoy DB, Guillemin GJ. The potential for transition metal-mediated neurodegeneration in amyotrophic lateral sclerosis. Front Aging Neurosci. 2014; 6:173.
34. Lahimer M, Abou Diwan M, Montjean D, Cabry R, Bach V, Ajina M, et al. Endocrine disrupting chemicals and male fertility: from physiological to molecular effects. Front Public Health. 2023; 11.
35. Musci G, Polticelli F, di Patti MCB. Ceruloplasmin-ferroportin system of iron traffic in vertebrates. World J Biol Chem. 2014; 5(2):204-15.