

Effects of Silver Nanoparticles on Lipid Peroxidation and Quality of Sperm Parameters in Male Rats

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J Babol Univ Med Sci; 18(2); Feb 2016; PP:48-55

Received: Apr 15th 2015, Revised: Jul 29th 2015, Accepted: Jan 6th 2016.

ABSTRACT

BACKGROUND AND OBJECTIVE: Considering the role of silver nanoparticles in inducing the production of free radicals, these compounds may lead to pathological effects and oxidative stress in the seminal fluid, resulting in the reduced quality of sperm parameters. Therefore, in this study, we aimed to assess the toxic effects of silver nanoparticles on sperm parameters and lipid peroxidation of sperm membranes in male rats.

METHODS: This experimental study was conducted on 24 Syrian adult male rats, which were randomly divided into one control and three study groups. The rats in the study groups were orally administered silver nanoparticles at concentrations of 0.07 µg (group A), 0.14 µg (group B), and 0.28 µg (group C), respectively per day for a period of five weeks. Then, lipid peroxidation of sperm membranes was analyzed, using thiobarbituric acid assay. Additionally, microscopic examinations were performed to evaluate sperm parameters.

FINDINGS: Based on the findings, the mean quality of sperm parameters such as sperm count and motility significantly declined in the study groups, particularly group B ($77.69 \pm 16.96\%$; $16 \pm 2.6 \times 10^6/\text{ml}$) and group C ($72.79 \pm 14.52\%$; $13.31 \pm 2.1 \times 10^6/\text{ml}$), compared to the control group (89.06 ± 10.97 ; $23.57 \pm 3.46 \times 10^6/\text{ml}$) ($p < 0.05$). Also, in terms of malondialdehyde concentration, a significant difference was reported among group A (0.25 ± 0.01), group B (0.32 ± 0.02), group C (0.35 ± 0.01), and the control group (0.24 ± 0.01) ($p < 0.001$).

CONCLUSION: The results of the present study confirmed that silver nanoparticles through enhancing lipid peroxidation could reduce the quality of sperm parameters in a dose-dependent manner.

KEY WORDS: Silver Nanoparticles, Sperm Parameters, Lipid Peroxidation, Male Rats.

Please cite this article as follows:

Layali E, Tahmasbpour E, Jorsaraei SGA. Effects of Silver Nanoparticles on Lipid Peroxidation and Quality of Sperm Parameters in Male Rats. J Babol Univ Med Sci. 2016;18(2):48-55.

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Introduction

In nanotechnology, use of silver nanoparticles is one of the most invaluable methods, with a wide range of applications in different fields such as biology and medicine (1). Silver nanoparticles possess antibacterial, antifungal, anti-viral, and anti-protozoan properties. Therefore, by using nanoparticles, we can create antimicrobial surfaces to eliminate infections (2). Nanoparticles are able to bind to the bacterial cell membrane and penetrate into the cells. The contributing factor for nanoparticle-membrane binding is the presence of sulfur proteins.

This binding not only leads to cellular death through altering the cellular morphology, membrane permeability, cellular respiration, and cell division, but also destroys the bacteria by penetrating into the cells and reacting with sulfur and phosphorus compounds (e.g., DNA) (1, 3-5). Silver nanoparticles can also affect viruses through binding to sulfhydryl groups of viral surface glycoproteins and prevent their adhesion to the host cell. In fact, silver nanoparticles can influence all types of DNA, RNA, coated, and uncoated viruses (6, 7). This advantage has increased the application of nanoparticles in the treatment of diseases with a bacterial origin.

Despite the advantages and wide application of nanoparticles, their toxicity and side-effects should be further evaluated and monitored. So far, some of the toxic effects of nanomaterials have been revealed, resulting in some limitations in the application of these compounds. Among these toxic nanomaterials, carbon and fullerene nanomaterials with five- and six-sided rings of carbon can be named (8-10). According to recent studies, globular or circular carbon structures have high water solubility. This property can result in serious damages such as oxidation of fatty acids in fish brain, which is in fact indicative of biological degradation (11-13).

Through enhancing the production of free radicals, single-walled carbon nanotubes are able to reduce the effective concentration of antioxidants in cells. In fact, metal contamination in the production stage of carbon nanotubes could add to their toxicity (13, 14). On the other hand, several studies have shown that physical

features of some nanotubes could be also involved in the development of pulmonary diseases and cancer (15-17). Due to their antimicrobial properties and production of free radicals, silver nanoparticles lead to oxidative stress and induce pathological effects in humans (18).

Many studies have shown that oxidative stress, caused by the increased production of free radicals and lipid peroxidation is one of the most important factors involved in the reduced performance and decreased quality of sperm parameters, which are closely related to infertility (19-25). Although several studies have demonstrated the negative impact of nanoparticles on different body organs, no comprehensive research has studied the effects of nanoparticles on the quality of sperm parameters, induced by the oxidation of sperm membrane lipids.

Considering the potential role of silver nanoparticles in enhanced production of free radicals, oversensitivity of sperm cells to the pathological effects of free radicals, and decreased level of antioxidants in the sperm cytoplasm, silver nanoparticles may be able to induce pathological effects, cause lipid peroxidation of sperm membranes, and consequently reduce fertilization.

Therefore, in this study, we aimed to evaluate the pathological effects of these nanoparticles on the level of lipid peroxidation and quality of sperm parameters in a rat model.

Method

Study samples: In this experimental study, 24 male rats were purchased from Pasteur Institute (Iran) and divided into one control and three study groups, each consisting of six rats. The rats were maintained under standard conditions, i.e., room temperature of 20-25°C, humidity of 55-60%, and a cycle of 12 hours of light and 12 hours of darkness. In a 35-day cycle of spermatogenesis, the rats were orally administrated different doses of silver nanoparticles via gavage on a daily basis. The study groups received 0.07 µg (group A), 0.14 µg (group B), and 0.28 µg (group C) of silver nanoparticle solutions, respectively (purity=99.99%;

size: 20 nm; Pishgaman of Nanomaterials, Iran). The control group only received normal food and water.

Sampling of epididymal sperm: After anesthetizing the rats, the abdominal area was sterilized by 70% ethanol and incised. Next, the abdominal fat layers were opened with the help of forceps. The end of the epididymis was cut using sterilized scissors and placed in Ham's F10 culture medium. By placing the epididymis in the growth medium, the sperm cells started to float towards the growth medium through gentle squeezing and shaking. Afterwards, the extra segments of epididymis were removed from the medium. The culture medium, containing the sperm suspension, was incubated at 37°C under 5% CO₂ pressure for 30 min. Following the incubation, the samples were studied in terms of sperm parameters. The suspension containing the sperms was transferred to microtubes, using a sampler micropipette. The samples were centrifuged at 1000 g for 10 min. The supernatant was removed from the samples and immediately stored at -70°C for later malondialdehyde (MDA) measurements.

Analysis of sperm parameters: In order to determine the percentage of sperms with normal morphology, Papanicolaou's staining method was applied in line with a study by Park and colleagues (26). To study sperm morphology, slides were prepared for each sample and then fixated. Afterwards, Papanicolaou's method was utilized for staining the samples. The percentage of motile sperms was measured, using methods applied in previous research (27-29). Afterwards, 10 µl of each sample was placed at the center of the Makler chamber. After placing the cover on the chamber, a light microscope was used to count the sperms at a magnification of X4. In this evaluation, a total of 100 cells were counted, and the percentage of sperm motility was determined according to the World Health Organization (WHO) standards (30). Moreover, sperm viability was determined via cytoplasmic method and eosin staining (31,32). Approximately 10 ml of semen specimens from all groups was placed on sterile slides and mixed with 10 ml of 5% eosin. After mixing the stain with the samples, sterile cover slips were placed on the slides, and the samples were

observed and measured using a light microscope at a magnification of X40. Considering the intactness of the cytoplasmic membrane, viable sperms could retain the eosin stain, which had penetrated into the cells; as a result, the sperms remained stained even after rinsing. On the contrary, non-viable sperms discarded the eosin stain due to their damaged membranes and appeared stainless under the microscope.

Measurement of lipid peroxidation of sperm membranes in the seminal fluid: In order to estimate the lipid peroxidation of sperm membranes, MDA concentration was measured in the seminal fluid of rats. MDA concentration was measured, using thiobarbituric acid reactive substance (TBARS) assay in line with a study by Tavilani and colleagues (33). Moreover, the MDA standard curve was depicted, using tetraethoxypropane (220.31 g/mol; Sigma Co.). Then, 100 ml of the examined samples was transferred to Eppendorf tubes and mixed with 500 ml of trichloroacetic acid. Subsequently, 10 ml of hydroxytoluene was added to the prepared solution and centrifuged at 3000 g for 10 min. Afterwards, 500 ml of the supernatant was removed from the solution, while adding 400 ml of TBARS (144.14 g/mol; Merck, Germany); the mixture was preserved at 95°C for one hour. The samples were stored and cooled down in a refrigerator for 15 min. Then, they were re-centrifuged at 4000 g for 10 min and the light absorption of the supernatant was analyzed by a spectrophotometer at a wavelength of 532 nm. Finally, MDA concentration was calculated, using the standard curve.

Statistical analysis: Comparison of the mean level of sperm parameters and MDA concentration was performed, using ANOVA and Least Significant Difference (LSD) tests. A p-value less than 0.05 was considered statistically significant.

Results

Results of the analysis of sperm parameters: Based on the findings, there was no significant difference in the mean body weight or weight of the left testicle in rats after 35 days. As the results indicated, group C showed the lowest mean sperm count compared to the

control group and study groups A and B. No significant difference was found in reduced sperm count between the control group and group A; however, the reduced sperm count in group B was significantly different from the control group (table 1). Compared to the control group, the lowest percentage of viable sperms was reported in groups B and C; also, the difference was statistically significant. As the findings revealed, the mean percentage of motile sperms in group C was significantly lower than other groups. On the other hand, the percentage of motile sperms in the control group was significantly higher than other groups. The highest percentage of immotile sperms was reported in groups B and C, compared to group A and the control group; however, there was no considerable difference between groups B and C. The percentage of sperms with abnormal morphology was higher in the study groups, particularly groups

receiving higher doses of silver nanoparticles. The highest and lowest percentage of viable sperms was reported in the control group and group C, respectively (table 1). The mean TBARS concentration in the seminal fluid was significantly different between the groups ($p < 0.001$). In fact, a rising trend was observed in the mean TBARS concentration from the control group to group C.

The mean TBARS concentration in group C (0.35 ± 0.01 nmol/ml) was significantly higher than the control group (0.24 ± 0.016 nmol/ml), group A (0.25 ± 0.01 nmol/ml) ($p < 0.001$), and group B (0.32 ± 0.02 nmol/ml) ($p < 0.05$). The mean TBARS concentration of the seminal fluid in group B was higher than the control group and group A ($p < 0.001$). However, the difference between group A and the control group was not significant in terms of TBARS concentration (fig 1).

Table 1. Comparison of sperm parameters among different groups

Parameters	Groups	Control Mean $\pm$ SD	A Mean $\pm$ SD	B Mean $\pm$ SD	C Mean $\pm$ SD	P-value
Number of samples		6	6	6	6	-
Administered dose of silver nanoparticles (μ g)		0	0.07	0.14	0.28	-
Body weight at the end of the study (g)		38.19 $\pm$ 4.25	35.14 $\pm$ 3.17	36.79 $\pm$ 2.41	34.31 $\pm$ 2.82	0.855
Weight of the left testicle by the end of the study (g)		0.12 $\pm$ 0.01	0.11 $\pm$ 0.02	0.11 $\pm$ 0.02	0.09 $\pm$ 0.01	0.399
Sperm count ($\times 10^6$ /ml)		23.57 $\pm$ 3.46	21.15 $\pm$ 3.02*	16 $\pm$ 2.6*	13.31 $\pm$ 2.1**	<0.001
Percentage of motile sperms (%)		89.06 $\pm$ 10.97	80.06 $\pm$ 9.83	77.69 $\pm$ 16.96**	72.79 $\pm$ 14.52*	<0.05
Percentage of immotile sperms (%)		12.3 $\pm$ 2.78	19.4 $\pm$ 5.16	22.31 $\pm$ 3.25*	27.21 $\pm$ 3.12*	<0.001
Viable sperms (%)		66.23 $\pm$ 5.13	57 $\pm$ 6.29**	51.4 $\pm$ 8.32**	7.51* $\pm$ 47.6	<0.01
Percentage of abnormal sperms (%)		28.17 $\pm$ 4.47	37.33 $\pm$ 1.27	35.83 $\pm$ 4.4**	41.67 $\pm$ 3.9*	<0.001

* $p < 0.01$, ** $p < 0.05$

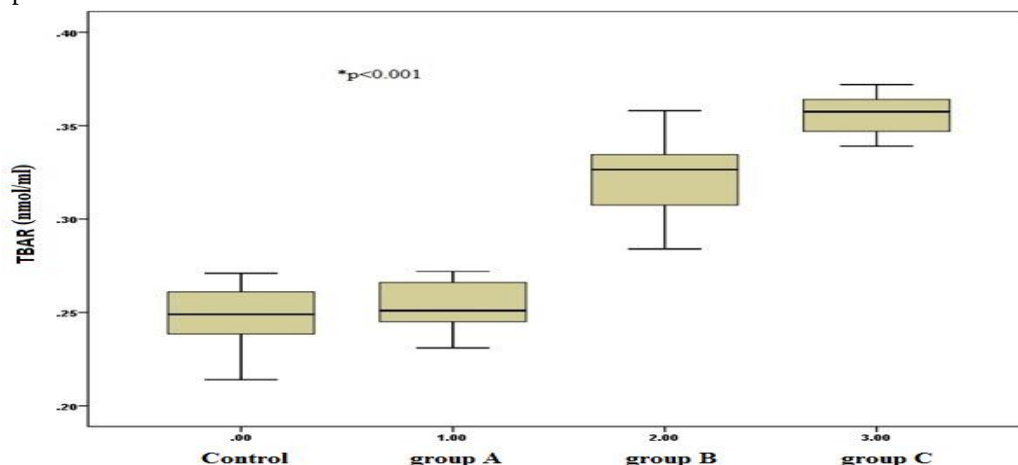


Figure 1. Comparison of the mean malondialdehyde (MDA) concentration between two groups. Group A: 0.07 μ g of silver nanoparticles, Group B: 0.14 μ g of silver nanoparticles, group C: 0.28 μ g of silver nanoparticles.

Discussion

Based on the analysis of sperm parameters, rats receiving silver nanoparticles showed a significantly lower sperm quality, compared to the control group. As a result, by increasing the dose of silver nanoparticles, the quality of sperm parameters, particularly normal morphology, viable sperm count, and percentage of motile sperms, decreased. On the contrary, the group receiving the highest concentration of silver nanoparticles (group C) showed the highest TBARS concentration, which could be indicative of the increased level of lipid peroxidation in rats. Based on the findings, silver nanoparticles not only could exert adverse effects on the quality of sperm parameters, but also could induce more severe pathological effects by increasing the concentration (dose-dependent properties).

It seems that one of the mechanisms involved in the effectiveness of silver nanoparticles in reducing sperm quality is enhanced oxidative stress, particularly lipid peroxidation, together with reduced effective concentration of antioxidants. Based on the current findings, it can be inferred that silver nanoparticles aggravate the adverse peroxidative effects in the seminal fluid, resulting in reduced sperm membrane fluidity and diminished sperm parameters. For this reason, the oxidative effects of silver nanoparticles and their pathological effects on the human body, especially on sperm quality and male fertility, should be assessed.

According to recent studies, silver nanoparticles at high doses (e.g., 200 mg/kg) can inhibit the clotting process by platelets and consequently increase the bleeding time. In addition, in previous research, the number of blood cells (i.e., red and white blood cells), hemoglobin concentration, as well as neutrophil and lymphocyte counts, has been reported to dramatically change (34-38).

Since few studies have examined the effects of nanoparticles (silver nanoparticles in particular) on fertility and sperm function, comparative analyses can be challenging. According to some previous studies, silver nanoparticles can block the growth of sperm cells and prevent sperm acrosome reactions (39-41).

Moreover, analysis of the effect of titanium oxide nanoparticles on fertility of mice showed that these nanoparticles could lead to a substantial decline in the number of Leydig cells, sperm viability, and expression of some genes through inducing oxidative stress (42). In another study, the results indicated an increment in DNA oxidation and morphological changes of seminiferous tubules in groups treated with silver nanoparticles. However, no changes were observed in these groups regarding the count of sperms with abnormal morphology (41). In a study by Baki and colleagues, the number of Leydig cells in the study groups (particularly the group receiving 200 mg/kg of silver nanoparticles) significantly reduced. Furthermore, the concentration of testosterone remarkably reduced, while the level of luteinizing hormones significantly increased; however, no significant change was reported in the concentration of follicle-stimulating hormones. On the other hand, there was a significant decline in the number, morphology, and percentage of motile sperms, which was consistent with the results of the present research (43).

Therefore, the effects of silver nanoparticles on the quality of sperm parameters and male fertility could be considered as a fundamental and alarming issue for future generations. As demonstrated in our study, oxidative status of the seminal fluid in the studied samples could be involved in minimizing sperm quality. Since silver nanoparticles could increase the production of free radicals through several mechanisms, the percentage of sperms with mutated DNAs was speculated to increase. This finding is of great significance in procedures such as embryo microinjection, considering the possibility of unsuccessful in vitro fertilization and even multiple damages in future.

With the development of different techniques, the majority of infertile men may overcome their infertility without facing any particular problems. However, sperms with an abnormal morphology (e.g., sperms mutated in one or more specific genes) may affect the in vitro fertilization of the egg (e.g., through intracytoplasmic sperm injection). This may either lead to unsuccessful fertilization or induce adverse side-

effects such as cancer in newborns and abnormal fetal development. Accordingly, evaluation of the application of silver nanoparticles in various scientific fields is of great significance. In fact, use of such technologies could boost men's reproductive abilities. The results of this study showed that silver nanoparticles could reduce the quality of sperm parameters in rats. One of the involved mechanisms in

this process might be increased lipid peroxidation of sperm cells. Moreover, the toxic effects of silver nanoparticles were shown to be dose-dependent.

Acknowledgments

Hereby, we would like to express our gratitude to those who assisted us with the laboratory experiments.

References

1. Wang Z, Xia T, Liu S. Mechanisms of nanosilver-induced toxicological effects: more attention should be paid to its sublethal effects. *Nanoscale*. 2015;7(17):7470-81.
2. Yang HL, Lin JC, Huang C. Application of nanosilver surface modification to RO membrane and spacer for mitigating biofouling in seawater desalination. *Water Res*. 2009;43(15):3777-86.
3. Xie W, Vu K, Yang G, Tawfiq K, Chen G. *Escherichia coli* growth and transport in the presence of nanosilver under variable growth conditions. *Environ Technol*. 2014;35(18):2306-13.
4. Wasowicz W, Cieslak M, Palus J, Stanczyk M, Dziubaltowska E, Stepnik M, et al. Evaluation of biological effects of nanomaterials. Part I. Cyto- and genotoxicity of nanosilver composites applied in textile technologies. *Int J Occupa Med Env Health*. 2011;24(4):348-58.
5. Yazdimamaghani M, Vashae D, Assefa S, Walker KJ, Madihally SV, Kohler GA, et al. Hybrid macroporous gelatin/bioactive-glass/nanosilver scaffolds with controlled degradation behavior and antimicrobial activity for bone tissue engineering. *J Biomed Nanotechnol*. 2014;10(6):911-31.
6. Seth D, Choudhury SR, Pradhan S, Gupta S, Palit D, Das S, et al. Nature-inspired novel drug design paradigm using nanosilver: efficacy on multi-drug-resistant clinical isolates of tuberculosis. *Curr Microbiol*. 2011;62(3):715-26.
7. El Khoury E, Abiad M, Kassaiy ZG, Patra D. Green synthesis of curcumin conjugated nanosilver for the applications in nucleic acid sensing and anti-bacterial activity. *Colloids Surf B Biointerfaces*. 2015;127:274-80.
8. Kumarathasan P, Breznan D, Das D, Salam MA, Siddiqui Y, MacKinnon-Roy C, et al. Cytotoxicity of carbon nanotube variants: a comparative in vitro exposure study with A549 epithelial and J774 macrophage cells. *Nanotoxicol*. 2015;9(2):148-61.
9. Khalid P, Hussain MA, Rekha PD, Arun AB. Carbon nanotube-reinforced hydroxyapatite composite and their interaction with human osteoblast in vitro. *Hum Exp Toxicol*. 2015;34(5):548-56.
10. Su J, Cao L, Li L, Wei J, Li G, Yuan Y. Highly sensitive methane catalytic combustion micro-sensor based on mesoporous structure and nano-catalyst. *Nanoscale*. 2013;5(20):9720-5.
11. Tsukahara T, Haniu H. Cellular cytotoxic response induced by highly purified multi-wall carbon nanotube in human lung cells. *Mol Cell Biochem*. 2011;352(1):57-63.
12. Shvedova AA, Pietroiusti A, Fadeel B, Kagan VE. Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress. *Toxicol App Pharmacol*. 2012;261(2):121-33.
13. Liu Z, Liu Y, Peng D. Carboxylation of multiwalled carbon nanotube attenuated the cytotoxicity by limiting the oxidative stress initiated cell membrane integrity damage, cell cycle arrestment, and death receptor mediated apoptotic pathway. *J Biomed Mat Res Part A*. 2015;103(8):2770-7.
14. Liu Y, Song W, Li W, Gaku I. In vitro cytotoxicity and oxidative damage effects of multi-wall carbon nanotube on RAW264.7 macrophages. *Wei Sheng Yan Jiu*. 2008;37(3):281-4.
15. Hilton GM, Taylor AJ, McClure CD, Parsons GN, Bonner JC, Bereman MS. Toxicoproteomic analysis of pulmonary carbon nanotube exposure using LC-MS/MS. *Toxicology*. 2015;329:80-7.
16. Dymacek J, Snyder-Talkington BN, Porter DW, Mercer RR, Wolfarth MG, Castranova V, et al. mRNA and miRNA regulatory networks reflective of multi-walled carbon nanotube-induced lung inflammatory and fibrotic pathologies in mice. *Toxicol Sci*. 2015;144(1):51-64.
17. Mitchell MJ, Castellanos CA, King MR. Surfactant functionalization induces robust, differential adhesion of tumor cells and blood cells to charged nanotube-coated biomaterials under flow. *Biomaterials*. 2015;56:179-86.
18. Hsin YH, Chen CF, Huang S, Shih TS, Lai PS, Chueh PJ. The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. *Toxicol Lett*. 2008;179(3):130-9.
19. Layali I, Tahmasbpour E, Joulaei M, Jorsaraei SG, Farzanegi P. Total antioxidant capacity and lipid peroxidation in semen of patient with hyperviscosity. *Cell J*. 2015;16(4):554-9.
20. Tahmasbpour E, Balasubramanian D, Agarwal A. A multi-faceted approach to understanding male infertility: gene mutations, molecular defects and assisted reproductive techniques (ART). *J Assist Reprod Gen*. 2014;31(9):1115-37.
21. Colagar AH, Marzony ET. Ascorbic Acid in human seminal plasma: determination and its relationship to sperm quality. *J Clin Biochem Nutr*. 2009;45(2):144-9.

- 22.Colagar AH, Marzony ET, Chaichi MJ. Zinc levels in seminal plasma are associated with sperm quality in fertile and infertile men. *Nut Res.* 2009;29(2):82-8.
- 23.Colagar AH, Jorsaraee GA, Marzony ET. Cigarette smoking and the risk of male infertility. *Pak J Biol Sci.* 2007;10(21):3870-4.
- 24.Hosseinzadeh Colagar A, Pouamir M, Tahmasbpour Marzony E, Jorsaraee GA. Relationship between seminal malondialdehyde and sperm parameters quality in the fertile and infertile men. *Braz Arch Biol Technol.* 2009; 52(6):1387-92.
- 25.Tahmasbpour Marzony E, Jorsaraei GH, Pouramir M ,Colagar AH. Seminal plasma antioxidant capacity in human semen with hyperviscosity. *Babol Univ Med Sci J.* 2012;14(16):39-44 .[In Persian]
- 26.Park YS, Park S, Ko DS, Park DW, Seo JT, Yang KM. Observation of sperm-head vacuoles and sperm morphology under light microscope. *Clin Exp Reprod Med.* 2014;41(3):132-6.
- 27.Kermani-Alghoraishi M, Anvari M, Talebi AR, Amini-Rad O, Ghahramani R, Miresmaili SM. The effects of acrylamide on sperm parameters and membrane integrity of epididymal spermatozoa in mice. *Eur J Obstet Gynecol Reprod Biol.* 2010;153(1):52-5.
- 28.Talebi AR, Khalili MA, Vahidi S, Ghasemzadeh J, Tabibnejad N. Sperm chromatin condensation, DNA integrity, and apoptosis in men with spinal cord injury. *J Spinal Cord Med.* 2013;36(2):140-6.
- 29.Mangoli E, Talebi AR, Anvari M, Pourentezari M. Effects of experimentally-induced diabetes on sperm parameters and chromatin quality in mice. *Iran J Reprod Med.* 2013;11(1):53-60.
- 30.Cardona Maya W. World health organization manual for the processing of human semen-2010. *Acta Urolog Espanol.* 2010;34(7):577-8.
- 31.Chalah T, Brillard JP. Comparison of assessment of fowl sperm viability by eosin-nigrosin and dual fluorescence (SYBR-14/PI). *Theriogenology.* 1998;50(3):487-93.
- 32.Cao XW, Lin K, Li CY, Yuan CW. A review of who laboratory manual for the Examination and Processing of Human Semen (5th edition). *Zhonghua nan ke xue.* 2011;17(12):1059-63.
- 33.Tavilani H, Doosti M, Saeidi H. Malondialdehyde levels in sperm and seminal plasma of asthenozoospermic and its relationship with semen parameters. *Clin Chimica Acta.* 2005;356(1-2):199-203
- 34.Taylor U, Tiedemann D, Rehbock C, Kues WA, Barcikowski S, Rath D. Influence of gold, silver and gold-silver alloy nanoparticles on germ cell function and embryo development. *Beilstein J Nanotechnol.* 2015;6:651-64.
- 35.Barkhordari A, Barzegar S, Hekmatimoghaddam H, Jebali A, Rahimi Moghadam S, Khanjani N. The toxic effects of silver nanoparticles on blood mononuclear cells. *Int J Occup Environ Med.* 2014;5(3):164-8.
- 36.Chen LQ, Fang L, Ling J, Ding CZ, Kang B, Huang CZ. Nanotoxicity of silver nanoparticles to red blood cells: size dependent adsorption, uptake, and hemolytic activity. *Chem Res Toxicol.* 2015;28(3):501-9.
- 37.De Jong WH, Van Der Ven LT, Sleijffers A, Park MV, Jansen EH, Van Loveren H, et al. Systemic and immunotoxicity of silver nanoparticles in an intravenous 28 days repeated dose toxicity study in rats. *Biomaterials.* 2013; 34(33):8333-43.
- 38.Drescher D, Buchner T, McNaughton D, Kneipp J. SERS reveals the specific interaction of silver and gold nanoparticles with hemoglobin and red blood cell components. *Phys Chem Chem Phys.* 2013;15(15):5364-73.
- 39.Miresmaeili SM, Halvaei I, Fesahat F, Fallah A, Nikonahad N, Taherinejad M. Evaluating the role of silver nanoparticles on acrosomal reaction and spermatogenic cells in rat. *Iran J Reprod Med.* 2013;11(5):423-30.
- 40.Mathias FT, Romano RM, Kizys MM, Kasamatsu T, Giannocco G, Chiamolera MI, et al. Daily exposure to silver nanoparticles during pre pubertal development decreases adult sperm and reproductive parameters. *Nanotoxicol.* 2015;9(1):64-70.
- 41.Gromadzka-Ostrowska J, Dziendzikowska K, Lankoff A, Dobrzynska M, Instanes C, Brunborg G, et al. Silver nanoparticles effects on epididymal sperm in rats. *Toxicol Lett.* 2012;214(3):251-8.
- 42.Komatsu T, Tabata M, Kubo-Irie M, Shimizu T, Suzuki K, Nihei Y, et al. The effects of nanoparticles on mouse testis Leydig cells in vitro. *Toxicol In Vitro.* 2008;22(8):1825-31.
- 43.Baki ME, Miresmaili SM, Pourentezari M, Amraei E, Yousefi V, Spenani HR, et al. Effects of silver nano-particles on sperm parameters, number of Leydig cells and sex hormones in rats. *Iran J Reprod Med.* 2014;12(2):139-44