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Comparative Analysis of the Effects of Magnesium Oxide Nanoparticles on Sperm Parameters in Fresh and Frozen Samples

Sheida Mirgalooye Bayat [1*†], Farahnaz Farzaneh [2*†], Shahla Mirgaloybayat [2]

1- Department of Biology, Islamic Azad University Science and Research Branch, Tehran, Iran
2- Department of Obstetrics and Gynecology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
† The first and the second authors have had equal contribution to this manuscript

Abstract

Background: Freezing is a crucial technique in reproductive science utilized for the preservation of sperm samples. However, the process of freezing and thawing sperm can result in detrimental effects on sperm quality. One of the major mechanisms underlying this decline in sperm quality is the generation of reactive oxygen species during the freeze process. The purpose of the current study was to investigate the effects of magnesium oxide nanoparticles on frozen sperm parameters.

Methods: Semen samples were collected from 8 fertile men, aged 30 to 42 years, with normozoospermia, following 3 to 5 days of abstinence. The samples were divided into fresh (n=3), freeze (n=3), and control (n=2) groups. Three fresh experimental groups were only exposed to MgO NPs with concentrations of 5, 25, and 50 *µg/ml* and three freezing experimental groups were frozen after being treated with MgO NPs, thawed, and analyzed after 30 *min*.

Results: Our findings revealed that the progressive movement and vitality of sperm experienced a significant decline, while non-progressive and immotile sperm showed a notable increase in both fresh and frozen experimental groups exposed to MgO NPs. However, the application of MgO NPs during fresh and freezing processes demonstrated an effective preservation of pH, morphology, and DNA fragmentation in sperm cells.

Conclusion: The analysis revealed that MgO NPs negatively impact sperm motility and viability in both fresh and freeze analysis. Also, the use of MgO NPs in fresh and frozen processes effectively maintains the pH, morphology, and fragmentation of DNA in sperm cells.

** Corresponding Authors:*
Sheida Mirgalooye Bayat, Department of Biology, Islamic Azad University Science and Research Branch, Tehran, Iran

Farahnaz Farzaneh, Department of Obstetrics and Gynecology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
E-mail:
sheidabayat777@yahoo.com,
f.farzaneh2019@gmail.com

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Introduction

Infertility, according to World Health Organization (WHO) definition, is a complex medical condition affecting the reproductive system, characterized by the inability to achieve pregnancy after a year of regular, unprotected sexual activity. While infertility may stem from various causes, male factors contribute significantly, accounting for approximately 44 to 55 percent of cases. Evaluation of male fertility typically involves assessing sperm count, motility, and morphology through semen analysis (1, 2). The cryopreservation of sperm is a crucial technique in reproductive medicine, particularly for individuals facing cancer treatments or other conditions jeopardizing fertility. Additionally, this technique is recommended for individuals with

non-malignant diseases, such as diabetes, autoimmune diseases, or high blood pressure, which can lead to testicular damage. It is also beneficial for those with a low number of sperm (3).

The sperm freezing technique can result in damage to sperm due to temperature variations and destructive changes in sperm structure, including the cell membrane, mitochondria, and DNA (4). To address this challenge, the addition of antioxidants or estrogenic compounds to the freezing environment has been explored to mitigate oxidative stress-induced damage.

Metal oxide nanoparticles, such as cerium oxide (CeO2) NPs, selenium (Se) NPs, and nano-zinc have emerged as promising agents for scavenging reactive oxygen species (ROS) and improving semen quality during cryopreservation. Among these nanoparticles, magnesium oxide nanoparticles (MgO NPs) hold particular interest due to their unique properties, including ease of synthesis, non-toxicity, affordability, and antimicrobial capabilities (5, 6).

Therefore, the addition of estrogenic compounds or potent antioxidants to the freezing environment may play a role in mitigating the risks associated with sperm freezing (2). The common materials in sperm-freezing medium are albumin and glycerol. This group of anti-freezing materials is more effective than water at low temperatures, consequently reducing the amount of ice formed inside the cell and minimizing damage caused by physical changes in cells and membranes (1).

Given that sperm cryopreservation could lead to a reduction in fertilization due to an increase of oxidative stress in spermatozoa, cerium oxide (CeO2) nanoparticles, nano-selenium and nano-zinc have also been used as scavengers of ROS which improve semen quality. Nanoparticles are currently utilized for increasing drug efficiency in the gastrointestinal tract, circulation, sperm function, and male fertility as antioxidants (7).

These nanoparticles are typically within the size range of 5 to 100 *nm* and have a specific surface area between 25 and 50 square meters per gram. Despite their small size, metal oxide nanoparticles exhibit significantly higher melting and boiling points compared to bulk materials. Their widespread use is attributed to their distinctive properties, including simple stoichiometry, crystalline structure, and high ionic conductivity (8).

The application of MgO NPs extends beyond sperm cryopreservation, with ongoing research demonstrating their potential in drug delivery, tumor targeting, and bioimaging. However, studies have also highlighted potential toxicity concerns associated with MgO NPs, including genotoxicity and oxidative stress-induced damage (9).

Ongoing clinical research on magnesium nanoparticles has yielded positive results. For instance, these nanoparticles provide precise drug delivery, enhanced permeability, and unique targeting to tumors with minimal or no side effects. Additionally, owing to their characteristics such as molecular weight, pH, ionic strength, and particle size, they are concurrently utilized in nano-cryosurgery, hyperthermia, tumor inhibition, and brain tumor imaging (5). A study has demonstrated that magnesium oxide nanoparticles induce significant toxicity, as evidenced by genotoxic, biochemical, histopathological, and biodistribution parameters. The study also revealed elevated levels of malondialdehyde and reduced glutathione, catalase, and superoxide dismutase, indicating the occurrence of oxidative stress. Consequently, the genotoxicity observed may be attributed to reactive oxygen species (ROS) (6). The results obtained from the study indicate that exposure to high doses of magnesium oxide nanoparticles causes DNA damage and significant biochemical changes (10). Therefore, the purpose of the current study was to investigate the effect of magnesium oxide nanoparticles on sperm parameters after freezing, aiming to fill a knowledge gap regarding their potential as protective agents in reproductive medicine. By elucidating the impact of MgO NPs on sperm quality, this research may contribute to advancing our understanding of their role in fertility preservation and future clinical applications.

Methods

Chemicals: Magnesium oxide nanoparticle powder with a diameter of 20 *nm* and a purity of 99% (CAS number 1309-48-4) was provided by Nanobazar, Iran. A solution of magnesium oxide was prepared by dissolving magnesium oxide in deionized distilled water as the solvent, with concentrations of 5, 25, and 50 *µg/ml* (Figure 1).

Semen collection and analysis: Semen samples were collected from 8 fertile men (n=8), aged between 30 and 42 years, exhibiting normozoospermia, after 3 to 5 days of abstinence. The samples were obtained from the Noor Pathobiology Laboratory in Tehran, Iran. Written informed consent was obtained from each subject before their inclusion in the study, and approval was granted by biomedical research ethics committee (IR.IAU.

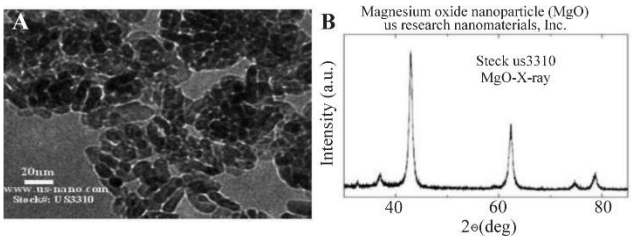

Figure 1. A) Transmission electron microscopy (TEM) image of magnesium oxide nanoparticle. B) X-ray refraction of magnesium oxide nanoparticles

SRB.REC.1401.317). Following the initial evaluation, samples with more than 10 million sperm per milliliter and a motility exceeding 42% were selected. Types of mobility, including A, B, C, and D were assessed by optical microscopy at 400×magnification and a light microscope equipped with the computer-assisted sperm analysis (CASA) system was utilized to classify the mobility according to 2010 WHO guideline, distinguishing between progressive, non-progressive, or immotile sperm. Additionally, factors such as vitality were determined using the trypan blue staining technique, pH measured with pH indicator paper, DNA fragmentation assessed using the sperm chromatin dispersion (SCD) method, and morphology analyzed through Diff-Quik (DQ) (IVFCO, Iran) staining.

Exposure procedure: Eight semen samples were divided into 8 fresh and freeze and control groups as follows (n=8):

1- Fresh control group: semen was analyzed 30 *min* after being placed in the environment.

2- Fresh experimental group: semen was exposed to a concentration of 5 *µg/ml* of magnesium oxide nanoparticles in a 1:1 ratio and then analyzed.

3- Fresh experimental group: semen was exposed to a concentration of 25 *µg/ml* of magnesium oxide nanoparticles in a 1:1 ratio and then analyzed.

4- Fresh experimental group: semen was exposed to a concentration of 50 *µg/ml* of magnesium oxide nanoparticles in a 1:1 ratio and then analyzed.

5- Freeze control group: the semen and freezing medium were frozen at the same concentration for a duration of 30 *min*. Subsequently, the samples were thawed and subjected to analysis.

6- Freeze experimental group: the semen was mixed with a freezing medium in a 1:1 ratio, along with a concentration of 5 *µg/ml* of magnesium oxide nanoparticles. The resulting mixture was then subjected to the freezing process and subsequently analyzed.

7- Freeze experimental group: semen was mixed with a freezing medium in a 1:1 ratio, along with a concentration of 25 *µg/ml* of magnesium oxide nanoparticles. The mixture was then subjected to the freezing process and subsequently analyzed.

8- Freeze experimental group: semen was mixed with a freezing medium in a 1:1 ratio, along with a concentration of 50 *µg/ml* of magnesium oxide nanoparticles. The mixture was then exposed to the freezing process and subsequently analyzed.

In all groups, analysis was conducted 30 *min* after freezing or adding nanoparticles.

Sperm freezing and thawing: In this study, magnesium oxide nanoparticles measuring 20 *nm* in powder form (Nanobazar Company, Iran) were utilized. For freezing, a freezing medium (Origio, Denmark) composed of glycerol, glucose, and HEPES (a buffering agent) in a 1:1 ratio was employed. To prepare the freezing solution, 1 *ml* of the freezing medium was added to 1 *ml* of the sample fluid. A volume of 250 *µl* of semen was mixed with 250 *µl* of the freezing medium. To initiate the freezing process, the sperm freezing medium was carefully added drop by drop onto the semen sample inside the cryotube along with a specific concentration of magnesium oxide nanoparticles in *µg/ml*, followed by gentle mixing. The mixture was left at room temperature for 15 *min*. Subsequently, the cryotubes were exposed to nitrogen vapor for 15 *min*, then transferred to liquid nitrogen at -196°*C* for 30 *min*. As a result, the samples were effectively frozen. After a 30 *min* cryostorage, the cryotubes were taken from the nitrogen tank, left at room temperature for 15 *min* for ice crystal thawing, and then immersed in standard tap water for an additional 15 *min* incubation. Following this process, the analysis resumed.

Sperm viability and motility: Initially, 50 *µl* of the semen sample were combined with 50 *µl* of trypan blue dye in a 1:1 ratio within a test tube. The mixture was then vigorously shaken for 10 *s*. Subsequently, 10 *µl* of the homogeneous sample were placed on a glass slide and examined using an optical microscope with a 40x lens. Sperm count was meticulously recorded using a counter. Sperm exhibiting a blue color with dye penetration in their head were classified as dead, while those without dye penetration were considered alive

(Figure 2). A total of 100 sperm were counted in each sample, and the percentage of live and dead sperm was meticulously documented.

Motility was assessed using the ocular motility testing with an optical microscope and the CASA system. Initially, 5 μl of the semen sample were carefully placed on a slide. Subsequently, the motion of sperm was precisely distinguished and documented using a microscope linked to CASA software, with a magnification of 20x. Following WHO guidelines, sperm were categorized as either progressive motile (moving linearly or in a large circle) or non-progressive. On the contrary, non-progressive sperm displayed diverse patterns of motion, including lateral movement, sluggish movement without forward progression, flagellar beating, circular movement, or complete immobility (8). Additionally, in the optical method, 5 μl of the semen sample were placed on a slide and re-examined with a microscope at 40x magnification from different angles.

Sperm DFI detection: DNA fragmentation was determined using the SDFA kit (Dain Bioassay, Iran), following the manufacturer's guidelines. Briefly, 50 μl of semen was diluted in Ham's F10 medium, and an aliquot was combined with 50 μl of 6.5% agarose. Next, 20 μl of the resulting mixture was applied to a pre-treated glass slide, which was then placed on a chilled surface at 4°*C* for 5 *min*. The slides were exposed to a denaturing solution for 7 *min*, followed by treatment with a lysing solution for 15 *min*. Afterward, the slides underwent a 5 *min* washing process with distilled water. Dehydration was achieved using ethanol at increasing concentrations of 70%, 90%, and 100%. Finally, the air-dried slide was stained. A minimum of 200 spermatozoa were observed and evaluated under a microscope with a 100x magnification. Spermatozoa with a large or medium halo were categorized as having intact chromatin, while those with either no halo or a small halo were classified as spermatozoa with fragmented DNA. The results were presented as the sperm DNA fragmentation index (DFI) (11).

Diff-Quik staining: Morphology detection was performed using a Diff-Quik kit (IVFCO, Iran). Initially, 10 μl of the specimen were carefully placed onto the slide, and the surrounding area was prepared. Subsequently, the slide was transferred to the incubator and left there until the semen had completely dried. Following the slide preparation, the staining process was carried out according to the manufacturer's guidelines. The slides were then left to dry in the environment or incubator, and morphology was meticulously examined using a magnification of 100x.

Figure 2. The evaluation of sperm viability. Trypan blue staining showing the heads of viable (white) and dead (blue) sperm, respectively (400x eyepiece magnification)

pH: In this research, to evaluate pH, a drop of seminal fluid was placed on pH indicator paper before adding nanoparticles to the semen liquid. The alkalinity or acidity was observed, and the pH was recorded. In the next step, after adding nanoparticles to the semen and following the freezing process, the pH was recorded in the same manner.

Statistical analyses: All sperm parameters are expressed as means±standard deviation (SD). Statistical analysis was conducted using a one-way analysis of variance (ANOVA) followed by a Dunnett post hoc test. This analysis was conducted to compare the fresh levels with the fresh control group and the frozen levels with the freeze control group. SPSS version 25 (IBM, USA) was employed for data analysis and visualization. The significance level was considered at p-value of <0.05.

Results

Semen pH assessment: In the comparison of the fresh experimental groups with the freeze groups, no significant changes in pH were observed, and there was no significant difference between the groups receiving MgO nanoparticles in fresh or freeze experimental groups and their respective control groups (Figure 3).

Sperm viability assessment: Regarding the viability parameter, the results indicated a decrease in the viability percentage in all concentration groups receiving MgO nanoparticles compared to both the fresh and freeze control groups. A significant difference was observed in the freeze experimental group receiving 50 $\mu g/ml$ MgO NPs compared,

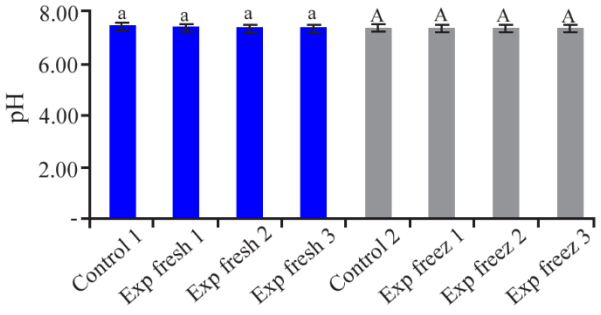

Figure 3. The trend of changes in pH parameters in different groups after 30 *min* in the environment (a) and 30 *min* after freezing (A). Small letters (a) represent fresh groups and capital letters represent freeze groups (A)

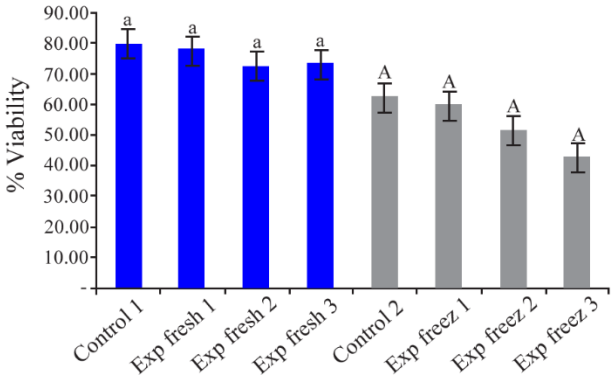

Figure 4. The trend of changes in sperm viability in different groups after 30 *min* in the environment (a) and 30 *min* following freezing (A). The non-uniformity of capital letters (A/B) indicates a significant difference between the freeze group and the corresponding control group (p<0/05)

with the freeze control group with a p-value less than 0.05 (Table 1 and figure 4).

Sperm motility assessment: A significant decrease in the percentage of progressive motility (A+B class) across all cohorts that received MgO NPs was noticed when compared to their respective control groups. There was a significant decrease in class A motility, in all concentrations of fresh and freeze experimental groups compared to both control groups (p<0.05). A significant difference was observed in class B motility between fresh and freeze experimental groups that received 50 *μg/ml* MgO NPs, compared to both the fresh and freeze control groups, with a p-value of <0.05. Regarding non-progressive motility (class C), a significant difference was observed in all concentrations of MgO NPs in the fresh experimental groups compared with the fresh control group and in concentrations of 25 and 50 *μg/ml* MgO NPs in the freeze experimental groups compared with the freeze control group (p<0.05). The immotile sperm percentage increased significantly in all concentrations of MgO NPs in the fresh experimental groups compared with the fresh control group and in concentrations of 50 *μg/ml* MgO NPs in the freeze experimental groups compared with the freeze control group (p<0.05) (Table 2).

Sperm morphology assessment: In the present study, the morphological defects of the sperm treated with MgO NPs were also observed before sperm freezing. The current images are related to sperm that were fresh and frozen-thawed and show that abnormal sperm still exist, and receiving MgO NPs had no positive or negative effect on sperm morphology compared to the control groups (Figure 5).

Sperm DNA fragmentation assessment: In the present study, the amount of DNA damage increased in the freeze experimental groups compared with the fresh experimental groups, but no significant increase was observed between the mean of DFI in the experimental fresh and freeze groups and their respective control groups (Figure 6).

Table 1. Descriptive viability statistics in fresh and freeze groups along with control groups at concentrations of 5, 25, and 50 *μg/ml* of Mgo NPs after 30 *min* in the environment and 30 *min* following freezing

Vit % (n=8)	Fresh Mean±SD	p-value [a]	Freeze Mean±SD	p-value [a]
Control	79±31	--	62±13	--
5 *μg/ml* Mgo (NPs)	77±9	0.991	59±9	0.929
25 *μg/ml* Mgo (NPs)	72±15	0.800	51±11	0.166
50 *μg/ml* Mgo (NPs)	72±16	0.844	42±12	0.005 *
p-value [b]	--	0.860	--	0.008 *

p-value a: Dunnett t-test was used to treat one group as a control and compare all other groups against it
p-value b: obtained from the ANOVA analysis
* Indicates statistical significance at p<0.05

Table 2. Descriptive motility statistics expressed as mean±standard deviations in both fresh and freeze groups along with control groups at concentrations of 5, 25, and 50 *μg/ml* of Mgo NPs after 30 *min* in the environment and 30 *min* following freezing

	Motility % (n=8)	Fresh Mean±SD	p-value [a]	Freeze Mean±SD	p-value [a]
Sperm progressive motility %	Control	27.1±13	--	0	--
	Class A (5 *μg/ml* MgO NPs)	9.4±10	0.012 *	0	0 *
	Class A (25 *μg/ml* MgO NPs)	11.1±10	0.025 *	0	0 *
	Class A (50 *μg/ml* MgO NPs)	9.2±12	0.011 *	0	0 *
	Class A p-value [b]	--	0.010 *	--	0 *
	Control	24.4±11	--	11.2±5.8	--
	Class B (5 *μg/ml* MgO NPs)	14.6±14	0. 173	7.1±4.1	0.190
	Class B (25 *μg/ml* MgO NPs)	11.6±8	0.053	6.8±5.1	0.155
	Class B (50 *μg/ml* MgO NPs)	10±7	0.027*	2.7±2.1	0.002 *
	Class B p-value [b]	--	0.045 *	--	0.009 *
Sperm non-progressive motility %	Control	4±1	--	18±5.3	--
	Class C (5 *μg/ml* MgO NPs)	1.3±1.5	0 *	15±2.6	0.266
	Class C (25 *μg/ml* MgO NPs)	0.5±0.7	0 *	13±3.7	0.039 *
	Class C (50 *μg/ml* MgO NPs)	0.8±0.7	0 *	10.6±3.2	0.002 *
	Class C p-value [b]	--	0 *	--	0.005 *
Immotile sperm %	Control	44.4±12.4	--	70.6±10.5	--
	Class D (5 *μg/ml* MgO NPs)	74.4±21	0.006 *	77.8±5.6	0.161
	Class D (25 *μg/ml* MgO NPs)	76.3±18	0.003 *	78.7±8	0.103
	Class D (50 *μg/ml* MgO NPs)	81±18.4	0.001 *	86.6±5	0.001 *
	Class D p-value [b]	--	0.001 *	--	0.003 *

p-value a: Dunnett t-test was used to treat one group as a control and compare all other groups against it
p-value b: Obtained from the ANOVA analysis
* Indicates statistical significance at $p<0.05$

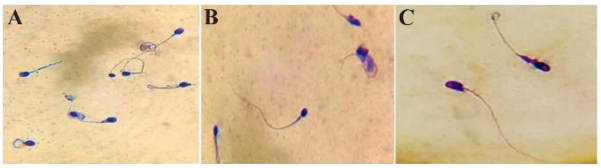

Figure 5. Morphology detection of sperm using Diff-Quik staining in various groups (1000x eyepiece magnification). A represents the fresh control group, B the freezing control group, and C the freezing + MgO NPs group

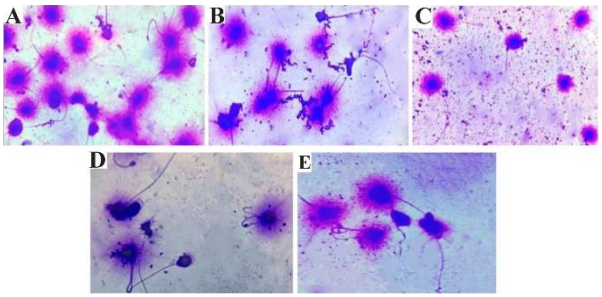

Figure 6. Human sperm DNA fragmentation analyzed by SCD assay in various treatment groups (1000x eyepiece magnification). A represents the fresh control group, B the fresh+ 5 *μg/ml* MgO NPs group, C the freezing+5 *μg/ml* MgO NPs group, D the freezing+25 *μg/ml* MgO NPs group, and E the freezing+50 *μg/ml* MgO NPs group

Discussion

It is important to note that comparable results were obtained in both the fresh and freeze experimental groups, indicating that the magnesium oxide nanoparticle functions under the same principles at room temperature and freezing conditions. However, it is worth mentioning that the frozen samples exhibited a more significant decrease in the measured parameters compared to the samples at ambient temperature. The widespread application of magnesium oxide nanoparticles can be attributed to their unique characteristics, such as easy synthesis, non-toxicity, affordability, and possession of antibacterial and antimi-

crobial properties (5). Antioxidants play a crucial role as the primary moderating factor between the physiological and pathological functions of reactive oxygen species (ROS). Essentially, antioxidants work to neutralize ROS, mitigating its active and aggressive nature, and inhibiting its involvement in the oxidative erosion process (12, 13).

The mitochondrial pathway of apoptosis is pivotal in the cell death induced by metal oxide nanoparticles, among various pathways that contribute to cell death. This is primarily attributed to the fact that mitochondria emerge as one of the principal organelles targeted by the oxidative stress induced by nanoparticles (14).

The main driver of reproductive toxicity induced by nanoparticles is oxidative stress (15). The primary cause of infertility issues in men stems from reactive oxygen species, as the increased production of ROS leads to cell apoptosis and impaired spermatogenesis (16). When nanoparticles are released into the environment, they have the potential to readily penetrate cells through receptor-mediated endocytosis or passive diffusion, interacting with cellular proteins, lipids, and genomic DNA (15).

Some nanoparticles have the potential to impact the reproductive system by disrupting protective tissues within the system, including epithelial, placental, and reproductive cells such as germ cells, Leydig cells, and Sertoli cells. Additionally, these nanoparticles can generate reactive oxygen species, serving as molecular mediators in the transmission pathways of spermatogenesis, steroidogenesis, and in the regulation of the hypothalamus-pituitary-gonadal axis. Consequently, this interference has implications for sperm maturation, as well as the occurrence of toxic effects such as DNA condensation and flagellum abnormalities (11).

The findings of the study indicated that the nanoparticle induces a cytotoxic impact on sperm. The introduction of magnesium oxide nanoparticles at concentrations of 5, 25, and 50 *μg/ml* drastically diminished progressive-type mobility both in its fresh condition and after freezing. A similar reduction was observed in non-progressive-type movement, or Class C, in the freezing groups subjected to concentrations of 50 and 25 *μg/ml* of the nanoparticle, exhibiting a significant decline compared to the freezing control groups. This reduction persisted in the subsequent experimental samples. In the case of immotile or class D, there was a significant increase in the concentrations of MgO nanoparticles compared to their control groups.

There was also a notable increase in the concentration of this nanoparticle in the freezing group compared to the corresponding control group. This nanoparticle exhibits a cytotoxic influence on spermatozoa, disrupting their motility. The results of this investigation align with previous findings (16), demonstrating the ability of this nanoparticle to induce dysfunction in the mitochondria and causing generation of reactive oxygen species by altering the potential of the mitochondrial membrane. Ultimately, this cascade of events leads to apoptosis and cell death.

In this investigation, no discernible adverse impacts on the structure, quantity, and pH characteristics were observed after the freezing- thawing process, both in the fresh experimental cohorts and in those treated with magnesium oxide nanoparticles. However, it is possible that increasing the nanoparticles concentration and prolonging the duration of exposure, both *in vitro* and *in vivo*, could potentially enhance their toxicity and result in more significant impairment in these parameters. The process of freezing and thawing intensifies harm to overall human health, as demonstrated by the increase in DNA damage and breakage in the freeze experimental groups compared to the fresh experimental groups. This aligns with the discovery that DNA induces irreversible changes in the well-being of the sperm's genetic material. Additionally, in both the fresh and freeze experimental groups exposed to a concentration of 25 *μg/ml* of magnesium oxide, the DNA breakage was higher than that caused by other doses derived from nanoparticles. However, this difference did not reach statistical significance. Regarding DNA breakage, the quantity of DNA damage and breakage in the freeze experimental groups exhibited an escalation compared to the fresh experimental groups. This discovery is in line with the findings of a study carried out by Fraser et al., (17) which provided evidence that the freezing-thawing process negatively impacts individuals' well-being. DNA induces irreversible alterations in the well-being of the sperm genome. Furthermore, in both the fresh and freeze experimental groups that received a concentration of 25 *μg/ml* of magnesium oxide compared to the fresh and freeze control groups, the extent of DNA breakage surpassed that induced by other doses of nanoparticles. However, it is noteworthy that this

disparity did not achieve statistical significance. Magnesium oxide nanoparticles belong to the category of metallic oxides that play a significant role in materials science and biomedical diagnostics due to their importance and extensive use. However, the potential toxicity of MgO nanoparticles towards bacterial and human cells, as well as organs, has not been thoroughly investigated. So far, the effect of magnesium oxide nanoparticles on semen parameters in an artificial environment, as well as the consequences after semen freezing, has not been studied in this way.

Several investigations have been conducted to examine the impact of MgO nanoparticles on different cell lineages. This effect may be associated with alterations in the mitochondrial membrane potential, which in turn triggers the phenomenon of apoptosis, ultimately resulting in cellular death (18). The findings derived from the research indicate that exposure to elevated levels of magnesium oxide nanoparticles leads to genomic DNA damage and notable alterations in the cellular biochemical profile (19).

Studies show that some nanoparticles may act as reproductive toxins which their effects depending on several factors, such as nanoparticle type, exposure pathway, and duration. These nanoparticles can cause damage to the male reproductive system by affecting structures such as sperm tubes and spermatozoa (20).

This is mainly because nanoparticles can easily enter the circulatory system and reach the testicles by crossing the blood-testis barrier. The biological accumulation of nanoparticles in the testicles causes histopathological changes in the seminiferous tubules semen-forming tubes and strongly affects the number, motility, and morphology of sperm (21). Additionally, it can cause reduced testosterone levels, resulting in testicular damage and a decrease in sperm production.

Since there has been no study of the effects of magnesium oxide nanoparticles on sperm, or at least no published results available, the findings of this study are only used to assess the risks of exposure to these nanoparticles.

In summary, our study elucidates the cytotoxic effects of magnesium oxide nanoparticles on human sperm parameters, highlighting their potential implications for reproductive health. The findings indicate a significant decrease in sperm viability and motility following exposure to these nanoparticles, with notable morphological changes observed under *in vitro* conditions. These results underscore the importance of understanding the dosage-dependent nature of nanoparticle toxicity and its impact on sperm functions. Moreover, our investigation sheds light on the mechanisms underlying nanoparticle-induced reproductive toxicity, emphasizing the role of oxidative stress and mitochondrial dysfunction in mediating cellular damage. While the study was conducted *in vitro*, further research is warranted to assess the safety and efficacy of magnesium oxide nanoparticles in clinical settings. Addressing the limitations of our study, including sample size and methodology, will be crucial in future investigations. Additionally, the clinical relevance of our findings in the context of fertility and reproductive health underscores the need for continued research to inform potential treatment strategies and regulatory measures. In fact, exploring unanswered questions and identifying future research directions will be essential for advancing our understanding of nanoparticle-mediated reproductive toxicity and its broader implications.

Conclusion

The findings of this study emphasize the potential toxic effects of magnesium oxide nanoparticles on human sperm parameters, in both fresh and frozen samples. Specifically, our results indicate a notable decrease in sperm viability and motility following exposure to these nanoparticles. It is important to emphasize that these observations were made under *in vitro* conditions, highlighting the need for further research to evaluate the safety and efficacy of this treatment approach in clinical settings. In addition to affecting sperm viability and motility, our study also identified morphological changes in sperm cells induced by magnesium oxide nanoparticles. While the exact nature and extent of these morphological alterations require further investigation, it is evident that *in vitro* conditions contribute to these changes. Future studies should aim to elucidate the specific morphological alterations and assess their consistency across both fresh and frozen samples. The concentration and duration of exposure to magnesium oxide nanoparticles emerged as critical factors influencing their toxicity on sperm cells. Our findings suggest a dosage-dependent nature of the nanoparticles' toxicity, highlighting the importance of carefully controlling exposure levels in clinical and experimental settings. Further research is needed to elucidate the precise relationship between exposure parameters and their effects on sperm pa-

rameters.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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