Advances in Nano Research, *Vol. 11, No. 6 (2021) 649-655*
https://doi.org/10.12989/anr.2021.11.6.649

In vitro effect of silver nanoparticles on avian spermatozoa

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(*Received October 16, 2020, Revised September 9, 2021, Accepted October 12, 2021*)

Abstract. Nanotechnology is widely considered a major technology of the twenty-first century. Nanoparticles (NPs) has been shown to pass through reproductively significant biological barriers such as the blood-testicle and placental barriers. Thus, the purpose of this study was to determine the effect of silver Nanoparticles (Ag-NPs) on sperm-egg interaction and spermatozoa quality parameters in quail spermatozoa. Semen was suspended in Ringer solution containing Ag-NPs levels at 5.5 × 106 sperm/ml (0, 0.01, 0.1, 1 and 10 ppm). The results indicated that when sperm were counted at 0.1 ppm, the number of holes formed on the inner perivitelline layer was significantly increased compared to the control. The 10 ppm group had a significant reduction in sperm viability. At 0.1 and 1 ppm, the membrane integrity was significantly decreased ($P < 0.05$). All treatments (except 0.01 ppm Ag-NPs) had a significant ($P < 0.05$) effect on the percentage of spermatozoa with an intact acrosome when compared to the control group. At 0.1, 1, and 10 ppm Ag-NPs, morphological defects in the acrosome were observed. As a result, Ag-NPs is likely capable of destroying the acrosome membrane. This research indicates that Ag-NPs may be cytotoxic to spermatozoa by impairing sperm functionality and increasing sperm mortality.

Keywords: acrosome; AgNPs; membrane integrity; quail; sperm-egg interaction

1. Introduction

The most widely used definition of NPs is materials designed and manufactured to have at least one dimension of 100 nanometers or less in their structural features (Oberdorster *et al*. 2005). Due to their unusual size, these materials exhibit novel physical, chemical, and biological properties that make them superior (Asare *et al*. 2012). Globally, the rapid advancement of nanotechnology has resulted in the massive production and use of engineered nanomaterials, even though the potential health consequences of these materials are largely unknown (Balasubramanian *et al*. 2010). Silver is considered to be more toxic than other metals. Ag-NPs was synthesized and demonstrated to be an effective antimicrobial agent through the advancement of nanotechnology due to its ability to bind to proteins and interfere with bacterial and viral processes (Kim *et al*. 2007). Ag-NPs is defined as nanoparticles (NPs) with a diameter of less than 100 nm (Abd AL-Rhman *et al*. 2016). Nanotoxicology is a relatively new branch of toxicology concerned with the safety of engineered nanostructures and nanodevices. Materials that are generally considered inert may exhibit unexpected behavior when introduced into the body as nanomaterials (Donaldson *et al*. 2004, Oberdorster *et al*. 2005). If Nanoparticles is absorbed into the body, toxicity screening strategies are required to determine the potential risk (Oberdorster *et al*. 2005). According to studies, the identical properties that distinguish Nanoparticles from other compounds may even be responsible for their potential toxicity. The majority of the effects are due to the high surface-to-volume ratio of the particles, which may make them highly reactive. Ag-NP's toxicity has been investigated in various cell types, including BRL3A rat liver cells (Hussain *et al*. 2005). In addition to macromolecules like proteins and DNA, NPs can bind to cells (AshaRani *et al*. 2009). When NPs come into contact with cells, they are absorbed via various mechanisms that can activate the cellular signaling process, resulting in the production of reactive oxygen species (ROS), inflammation, and, finally, cell cycle arrest or necrobiosis (AshaRani *et al*. 2009). Apoptosis is thought to be induced by excessive ROS production in the cell. ROS generation has been shown to be critical in inducing apoptosis by Ag-NPs treatment (AshaRani *et al*. 2009, Foldbjerg *et al*. 2009). Reduced proliferation rate, impaired mitochondrial function, and induction of apoptosis or necrosis are among the changes observed in cells exposed to Ag-NPs (AshaRani *et al*. 2009). Additionally, a decrease in cell viability was observed in Ag-NPs-treated liver and nerve cells (Hussain *et al*. 2005). Following the induction of DNA damage or the inhibition of cellular processes, these events may form a mutant or tumorigenic cell. Because Ag-NPs readily binds to proteins and glycoproteins, it is necessary to evaluate the Nanoparticles's interactions with tissues and cells (Braydich-stolle 2010). Furthermore, prior research indicates that NPs penetrate the blood-brain barrier and are distributed in the gonads after crossing the blood-testicular barrier (McAuliffe and Perry 2007, Yoshida 2008, De Jong 2009, Takeda 2009, Braydich-stolle 2010). When germline cells are exposed to such processes, the result may be altered spermatogenesis and fertility, subsequently affecting the offspring's reproduction rate and health (Ema *et al*. 2010).

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ISSN: 2287-237X (Print), 2287-2388 (Online)

However, Nanoparticle exposure in somatic cells can result in inflammation or even malignant transformation, whereas reproductive toxicants directed at the germline can result in changes that genetic or epigenetic mechanisms will pass on to the next generation (Asare *et al*. 2012). Although there is growing concern regarding the potential effects of NPs on reproductive health, Ag-NPs is one of the more recent products used in the poultry industry as an antimicrobial, specifically an antiseptic or disinfectant and sterilant. However, no studies on avian sperm have been conducted to date. As such, this study will examine the effects of Ag-NPs on sperm cells using quail as a model animal.

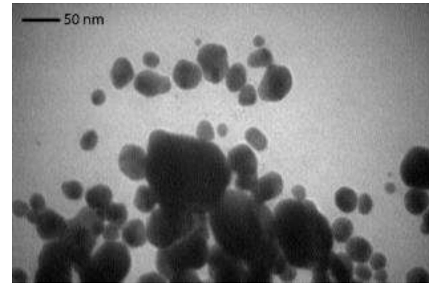

Fig. 1 TEM image of AgNPs, Abbreviations: TEM, transmission electron microscopy, AgNPs, silver nanoparticles

2. Material and methods

2.1 Chemicals and AgNPs preparation

The colloidal Ag-NPs (Nanocid L2000, Concentration 4000 mg/L, Nano Nasb Pars Company, Tehran, Iran) was synthesized via a novel process involving the photo-assisted reduction of Ag+ to metallic Nanoparticles, registered under United States Patent Application No: 20090013825. The particle size and shape of the applied colloidal Ag-NPs samples were confirmed through a transmission electron microscope (TEM) Philips CM120 (FEI Co, Eindhoven, The Netherlands) (Fig. 1). At Kefa Nano Laboratory (KNL), Tehran, Iran, the particle size distribution and zeta potential of silver particles in colloid were determined using dynamic light scattering (DLS) (Zetasizer ZS, Malvern Instruments Ltd, Worcestershire, UK). After nitric acid digestion, the solution's silver concentration was determined using graphite furnace atomic absorption spectrometry (GFAAS) (Phoenix-986, Biotech Engineering Management, U.K.).

2.2 Birds, farming conditions

All procedures used in this study were approved by the Animal Care and Use Committee of the University of Kurdistan (Sanandaj, Iran), with permission number 901181/28.9.1390. Throughout the experiments, mature Japanese male and female quail were used. They were caged, separated, and fed ad libitum on a commercial breeder's ration (22% crude protein and 3050 kcal ME/kg).

2.3 Semen collection and processing

After decapitation of a male quail, semen was collected via syringe from the distal cauda of the vas deferent (n = 6, replicates). The volume and sperm concentration of each bird's sperm were determined using graduated collecting tubes and a Neubauer hemocytometer. Sperm forward and total motility were determined using a diluted sample (1:20 semen to Ringer solution), they were then placed on a cover slide and subjectively analyzed on a warm stage (37°C) using a light microscope (Olympus, Japan) at a magnification of 100. The experiment used semen samples with high motility (85%). Each bird's sperm was isolated and suspended at a concentration of 5.5×106 sperm/ml in Ringer solution containing Ag-NPs levels (0, 0.01, 0.1, 1, and 10 ppm). The sperm concentration was determined to be adequate for creating the most significant number of countable holes in the IPVL (Win *et al*. 2006). Experiments were repeated three times in each replicate for each level.

2.4 Preparation of Inner Perivitelline Layer (IPVL)

Freshly laid eggs that had not been fertilized were collected from females between 15 and 30 weeks. The eggs were then cracked open. Following that, egg yolks were isolated and washed in 1% NaCl to remove albumen. After immersing the yolk in PBS, it was incubated at 37°C for 1 h. Following incubation, the PBS was removed and replaced with 1% NaCl. The yolks were placed in a petri dish on top of the blastoderm. Forceps were used to puncture the yolk. Around the egg's equator, the obtained perivitelline layer was cut away, and the non-germinal disc region was removed. The inner layer of the vitelline membrane was manually separated from the outer layer by repeatedly washing the membrane in 1% NaCl.

2.5 In vitro sperm penetration into IPVL

The in vitro sperm-egg interaction assay accurately reflects spermatozoa's fertilizing ability and more sensitively detects spermatozoa damage caused by toxicants than motility or fertility tests (Kasai *et al*. 1994). The sperm penetration assay was performed in laid eggs by fixing and staining the intact IPVL section with Schiff's reagent to observe the effects of Ag-NPs on sperm functionality. IPVL was divided into two pieces of approximately 0.5×0.5 cm^2, one for control and the other for treatment. This experiment utilized a pair of test tubes. In a control tube, sperm suspension was contained. The remaining tubes contained sperm and a corresponding concentration of Ag-NPs. Layers were incubated at 39°C for 40 minutes in microtubes with a 100 μl suspension of 5.5×10^6 sperm/ml in Ringer solution containing 0.01, 0.1, 1, and 10 ppm Ag-NPs. After incubation, the reaction was terminated by placing the tube at 4°C, and the IPVL was washed three times with ice-cold PBS. After fixation with 20% (v/v) formaldehyde, the IPVL was positioned on a glass slide and stained with Schiff's reagent. A light microscope was used to count the holes formed on the IPVL in the 40× field. Ten areas were randomly chosen to count perforations. This experiment was repeated six times. Fig. 2 summarizes the steps involved in sperm-egg interaction.

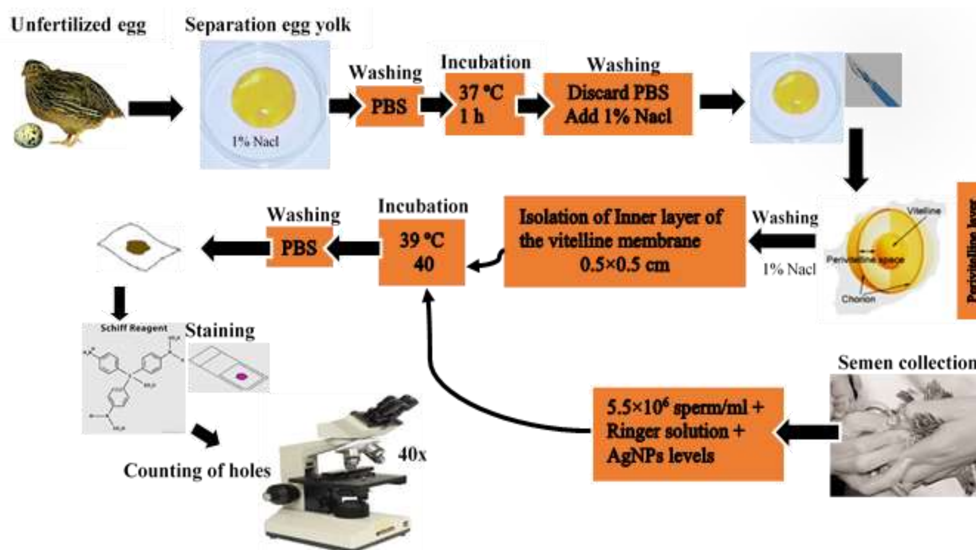

Fig. 2 Summary of flow diagram for sperm-egg interaction

2.6 Quality assays of spermatozoa

2.6.1 Viability

The sperm viability and incidence of normal and defective sperm were determined after 40 min incubation with AgNPs using the eosin-nigrosin test. Forty microliters of semen samples of each treatment were added to 150 µl of the staining solution (eosin 16 g/l and nigrosin 60 g/l in Ringer solution) and put onto a slide. Two minutes later, smears were performed for each sample, and spermatozoa were observed, using a microscope with an oil immersion objective. Live spermatozoa, eosin-impermeable, were seen white in color. In contrast, dead spermatozoa were color-pink due to their eosin-permeable. 300 spermatozoa were observed for each sample and the percentage of live/dead and defective sperm was calculated (El-Gendy *et al*. 2007).

2.6.2 Membrane integrity

The hypo-osmotic swelling test was used to determine the sperm membrane's integrity. To determine membrane integrity, 25 µl of diluted semen was combined with 500 µl of hypoosmotic solution (100 mOsm/kg) prepared by adding 1 g sodium citrate ($Na_3C_6H_5O_7$) to 100 ml distilled H2O, the test was also performed at lower incubation temperatures (laboratory temperature: approximately 23°C). The coiled mid-pieces and tail segments of 300 spermatozoa were examined under a light microscope (M-400x) (Santiago-Moreno *et al*. 2009).

2.6.3 Acrosome integrity

The wet mount of diluted spermatozoa fixed in buffered Formalin-Citrate solution was used to assess morphological acrosome abnormality, as described by Farshad *et al*. (2009). The fixed spermatozoa were then placed in a drop on a slide and covered with a cover glass. A BX51 microscope equipped with an M-600 objective and white light was used to examine the slides. The percentage of spermatozoa with normal acrosomes was determined by examining spermatozoa (n = 300 per slide).

3. Statistical analysis

The data were analyzed using the SAS general linear model (GLM) procedure in a completely randomized design. Duncan's multiple range test (MRT) was used to determine the statistical significance of differences between means, and a probability level of less than 0.05 was considered statistically significant.

4. Results

The TEM image was analyzed to determine the average size of Nanoparticles. The TEM images in Fig 1 indicate that the mean particle size of Ag-NPs was <50 nm.

Fig 2 shows a summary of flow diagram for sperm-egg interaction.

Abundant holes (Fig. 3) were observed in the IPVL of the AgNPs-treated groups as compared with the control group ($P \leq 0.05$).

Means for Viability, Acrosome integrity, membrane integrity (%) for spermatozoa after incubation (30 min) with or without the presence of AgNPs are shown in Fig 4.

There were significant treatment effects (0.1, 1, and 10 ppm AgNPs) on viability (Fig. 6), acrosome integrity, and membrane integrity (Fig. 7). Incubation of sperm with the 1 ppm AgNPs was associated with a significant ($P < 0.05$) decline of integrity (%) as compared with the control and 0.01 ppm of AgNPs groups. The acrosome integrity (%) for control and 0.01 ppm AgNPs groups were 87.2 and 76.9, respectively. In contrast, the acrosome integrity in the 0.1, 1, and 10 ppm of AgNPs-treated groups were 68.3, 70.6, and 69.2, respectively. These values were significantly lower than that of the control group ($P < 0.05$). Similar values of sperm viability were also obtained for control and 0.01, 0.1, and 1 ppm AgNPs groups. The mean value of sperm viability for the 10 ppm AgNPs group was 51.8% that was dramatically reduced as compared with other groups ($P < 0.05$). No significant differences in motility of

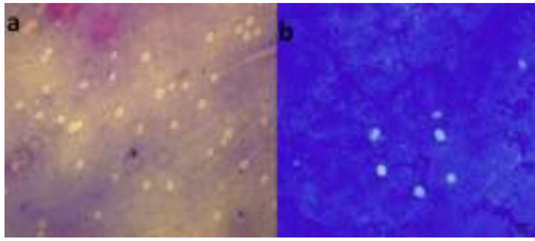

Fig. 3 Digital micrographs show in vitro formation of holes on the surface of the inner Perivitelline layer by quail sperm (5×10^6 cells/ml) in the presence (a) or absence (b) of AgNPs (M-100 x)

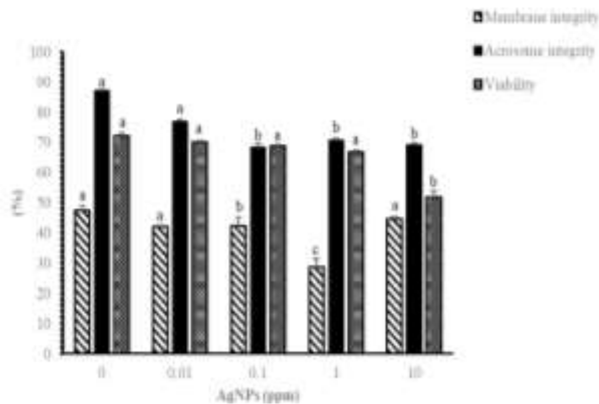

Fig. 4 The effects of AgNPs on the various sperm functional parameters

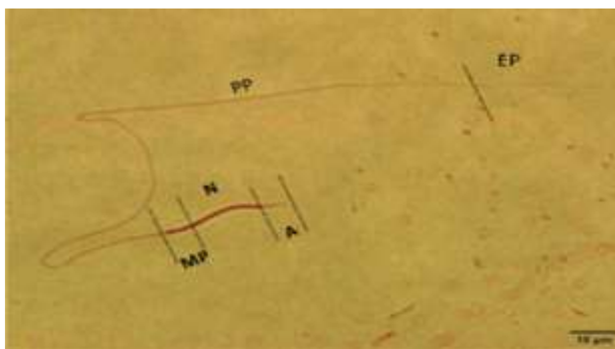

Fig. 5 Quail spermatozoa (normal). A, acrosome, N, nucleus, MP, midpiece, PP, principal piece, EP, endpiece.Sperm stained with Eosin nigrosin.Scale bar = 10 µm

spermatozoa were observed between experimental groups (Table 1). Fig 5 shows a light microscopy of normal quail spermatozoa. Avian sperm showing a cylindrical shape divided in head, midpiece, and tail (Fig. 5).

The head has a very evident acrosome, the tail consists of a main piece and end piece. Abnormal sperms were increased significantly in AgNPs groups ($P < 0.05$) after incubation of quail sperm with 10 ppm of AgNPs as compare with other groups. Defective spermatozoa were divided into two broad categories, namely, head defects, tail defects. Head defects include coiled head, bent acrosome, spiral acrosome, broken head, hookless, bent neck, knotted head, and detached head.

Tail defects include broken midpiece, bent tail, strongly folded, knotted tail, broken tail, coiled tail, detached tail (Fig. 8).

Fig. 6 Detailed differential sperm morphology evaluation in a semen sample stained with Eosin-nigrosin observed at x 1000 magnification/oil immersion with light microscopy

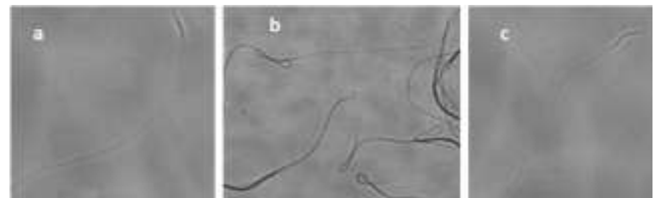

Fig. 7 Avian swollen-tail spermatozoa after subjection to hypo-osmotic solution (×1000); Straight sperm, damaged plasma membrae (a and c); Coiled sperm, intact plasma membrane e (b)

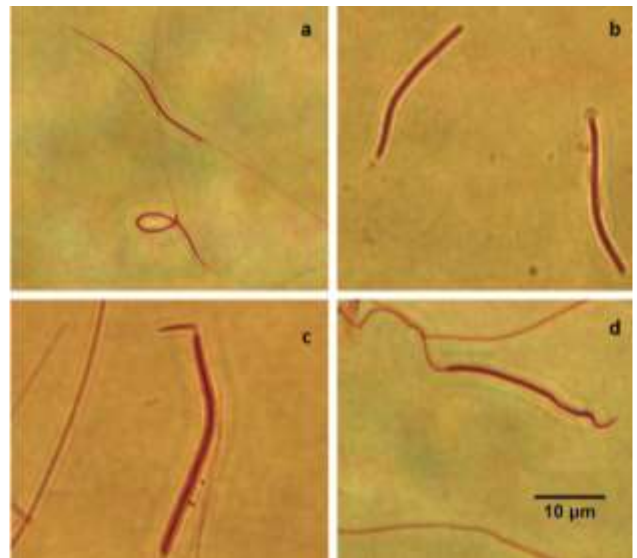

Fig. 8 Representative images of morphological defects of AgNPs-treated spermatozoa; (a) Coiled head, (b) detached head, (c) bent acrosome, (d) spiral acrosome. Magnification ×1000

The results indicate that AgNPs can induce sperm abnormalities, decrease sperm numbers if take at high doses (Table 1, $P < 0.05$).

5. Discussion

Reproductive toxicity of nanomaterials has become a critical component of nanotoxicology (Ema *et al.* 2010).

Table 1 Motility and incidence of spermatozoa defects determined from the stained semen smears (Three hundred spermatozoa per sample were observed)

Sperm morphology	AgNPs (ppm)				
	0	0.01	0.1	1	10
Motility of Spermatoza (%)	29	31	27	30	23
Head defects (%) *	4.9	6.3	10.5	9.7	10.5
Tail defects (%) **	3.3[b]	4.1[b]	4.6[b]	2.8[b]	11.2[a]
Total abnormal spermatozoa (%)	8.2 [b]	10.4 [b]	15.1 [b]	12.5 [b]	21.7[a]

The different small letters indicate that there were significant differences at value of $P \leq 0.05$
*Head defects include coiled head, bent acrosome, spiral acrosome, broken head, hookless, bent neck, knotted head and detached head.
**Tail defects include broken midpiece, bent tail, strongly folded, knotted tail, broken tail, coiled tail, detached tail.

The primary objective of this study was to determine the toxicity of different Ag-NPs concentrations on avian spermatozoa. Three parameters were used in toxicological studies: viability, cell membrane integrity, and acrosomal integrity. Additionally, the sperm-IPVL assay was used to determine the effects of Ag-NPs on spermatozoa fertilization ability. Sperm must be motile, capacitated, and capable of undergoing the acrosome reaction to fertilize the egg. Following the spermatozoon's attachment to the egg, it undergoes the acrosome reaction, which allows the sperm to penetrate the egg and fertilize it (Makhluf *et al.* 2008).

The interaction of spermatozoa was disrupted, most likely by the disappearance of the sperm cells' acrosomal membrane. After 40 minutes of incubation, the effect of the Ag-NPs on the quality characteristics of the sperm cells was determined through the following set of experiments. The results indicate that the proportion of intact acrosomes was statistically decreased at 0.1, 1, and 10 ppm Ag-NPs concentrations, while the membrane was significantly damaged at 0.1 and 1 ppm. The first barrier is the cell membrane, which is selectively permeable and can regulate what enters and exits the cell (Makhluf *et al.* 2008). As a result, this fraction is at risk of being harmed. Inadequate actual information regarding the effects of NPs and other NMs on sperm functionality in avian and mammalian sperm precludes an adequate discussion of the study's findings.

Despite the blood-testicular barrier, Kwon *et al.* (2008) reported on the penetration of inhaled magnetic nano-particles into the testis. Additionally, it has been demonstrated that intravenously injected Ag-NPs reach the testis (Lankveld *et al.* 2010). Furthermore, Ag-NPs administered intravenously to rabbits at a dose of 0.6 mg/kg BW resulted in significantly less motile sperm than control (Castellin *et al.* 2014). Their study shows that Ag-NPs can reach the testes, impairing sperm motility, sperm speed, the shape and function of the acrosome, and mitochondria. In another study, Fathi *et al.* (2019) reported a significant decrease in sperm count ($p < 0.0001$), vitality ($p < 0.05$), and morphology changes ($p < 0.001$) in the group receiving 300 mg/kg Ag-NPs. Moreover, Asare *et al.* (2012) recently demonstrated that Ag-NPs is particularly toxic to testicular cells. Other studies have also confirmed the adverse effects of NPs (Farzinpour and Chobdarian 2012, Farzinpour and Karashi 2013, Rezaei *et al.* 2018). In vitro studies on the reproductive and developmental effects of manufactured NPs revealed that they cause necrosis, apoptosis, and various mitochondrial dysfunctions in cells (Wiwanitkit *et al.* 2009, Braydich-Stolle *et al.* 2010). So far, evaluations of sperm toxicity have been conducted using Nanoparticles derived from silver (Moretti *et al.* 2012) and gold (Wiwanitkit *et al.* 2009, Taylor *et al.* 2010, 2013, Zakhidov *et al.* 2012, Moretti *et al.* 2012). While Fe_3O_4 particles had no detrimental effect on sperm, AuNP decreased sperm motility and increased sperm fragmentation (Taylor *et al.* 2010, Abdolvanl *et al.* 2020, Wiwanitkit *et al.* 2009). As a result, the effect observed may be due to byproducts of the chemical fabrication process or the stabilizing agent rather than the NPs themselves. Similarly, in vivo studies corroborate the in vitro findings and suggest that the deleterious effects of NPs are proportional to their chemical composition, size, and dosage (Takeda *et al.* 2009). Nanoparticles tends to be toxic to cells involved in male reproduction. Yoshida *et al.* (2008) demonstrated that different sizes of carbon Nanoparticles (black) (14, 56, and 95 nm) could cause harm to the male reproductive system in mice. Notably, according to the authors' histological observations, these Nanoparticles can result in partial vacuolization of the spermatogenic epithelium, resulting in the formation of additional niche-forming Sertoli cells. Furthermore, carbon Nanoparticles reduces daily sperm cell production but significantly increases serum testosterone synthesized by Leydig cells. Similar experiments on the effect of Nanoparticles or titanium oxide (TiO_2) or Ferrum oxide (Fe_3O_4) on pregnant female mice produced significant destructive changes in the structure of seminiferous tubules, including a decrease in the number of spermatogonia, primary spermatocytes, spermatids, and sperm cells (Takeda *et al.* 2009). In rats, silica oxide (SiO_2) Nanoparticles caused severe histopathological damage to the testes, a decrease in the number of sperm cells, and a decrease in lactate dehydrogenase-C4 activity (Fan *et al.* 2006). Without a doubt, specific nanomaterials are toxic to a variety of tissues, cells, and organelles. Numerous parameters relating to Nanoparticles, including its form, size, charge, and other physical and chemical properties, as well as its dosage and route of administration, should be considered when discussing the present study's findings.

6. Conclusions

This study concluded that Ag-NPs might impair sperm

functionality by causing damage to the plasma membrane, particularly the acrosome membrane. The data established Ag-NP's toxicity on spermatozoa, implying that Ag-NPs-treated cells were damaged. Subsequently, these cells' ability to bind to and induce an acrosome reaction to penetrate an egg was impaired compared to control cells, indicating that this critical function of the sperm is disrupted following NP treatment. The in vitro results established that the danger posed by Ag-NPs is dependent on their chemical composition, size, dosage, route of administration, and duration of exposure. Further research is necessary to elucidate the mechanisms underlying NP-induced male reproductive dysfunction. In general, caution should be exercised when using Ag-NPs in poultry farms due to its potential toxicity to spermatozoa cell functionality.

Acknowledgments

We thank the members of the Chemistry Laboratory at the College of Basic Science, University of Kurdistan, Kurdistan, Sanandaj. Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

Disclaimer

All authors have read the manuscript and agreed to submit it in its current form for consideration for publication in the journal.

References

Abd AL-Rhman, R.M., Ibraheem, S.R. and Israa, A.O. (2016), "The effect of silver nanoparticles on cellular and humoral immunity of mice in vivo and in vitro", *Iraqi J. Biotechnol.*, **15**(2), 21-29.

Abdolvand, E., Farzinpour, A. and Vaziry, A. (2020), "Effects of supplementation cysteine-coated Fe_3O_4 nanoparticles compared to FeSO4, on reproductive performance in male quail", *Adv. Nano Res.*, **9**(1), 15-24. https://doi.org/10.1080/1828051X.2017.1345662.

Ahamed, M., Posgai, R., Gorey, T.J., Nielsen, C.M., Hussain, S.M. and Rowe, J.J. (2010), "Silver nanoparticles induced heat shock protein oxidative stress and apoptosis in Drosophila melanogaster", *Toxicol. Appl. Pharm.*, **242**(3), 263-269. https://doi.org/10.1016/j.taap.2009.10.016.

Asare, N., Instanes, C., Sandberg, W.J., Refsnes, M., Schwarze, P., Kruszewski, M. and Brunborg, G. (2012), "Cytotoxic and genotoxic effects of silver nanoparticles in testicular cells", *Toxicology*, **29**(1-3), 65-72. https://doi.org/10.1016/j.tox.2011.10.022.

AshaRani, P.V., Hande, M.P. and Valiyaveettil, S. (2009), "Anti-proliferative activity of silver nanoparticles", *BMC. Cell. Biol.* **10**(65), 461. https://doi.org/10.7314/APJCP.2015.16.14.6039.

Balasubramanian, S.K., Jittiwat, J., Manikandan, J., Ong, C.N., Liya, E.Y. and Ong, W.Y. (2010), "Biodistribution of gold nanoparticles and gene expression changes in the liver and spleen after intravenous administration in rats", *Biomaterials*, **31**(8), 2034-2042. https://doi.org/10.1016/j.biomaterials.2009.11.079.

Braydich-Stolle, L.K., Lucas, B., Schrand, A., Murdock, R.C., Lee, T., Schlager, J. and Hofmann, M.C. (2010), "Silver nanoparticles disrupt GDNF/Fyn kinase signaling in spermatogonial stem cells", *Toxicol. Sci.*, **116**(2), 577-589. https://doi.org/10.1093/toxsci/kfq148.

Castellini, C., Ruggeri, S., Mattioli, S., Bernardini, G., Macchioni, L., Moretti, E. and Collodel, G. (2014), "Long-term effects of silver nanoparticles on reproductive activity of rabbit buck", *Syst. Biol. Reprod. Med.*, **60**(3), 143-150. https://doi.org/10.3109/19396368.2014.891163.

De Jong, W.H., Hagens, W.I., Krystek, P., Burger, M.C., Sips, A.J. and Geertsma, R.E. (2008), "Particle size-dependent organ distribution of gold nanoparticles after intravenous administration", *Biomaterials*, **29**(12), 1912-1919. https://doi.org/10.1016/j.biomaterials.2007.12.037.

El-Gendy, E.A., Gad, A.Y. and Mostageer. A. (2007), "Sperm-mediated gene transfer in poultry. 1. The relationship with cock sperm viability", *Arab. J. Biotechnol.* **10**(1), 1-12.

Ema, M., Kobayashi, N., Naya, M., Hanai, S. and Nakanishi, J. (2010), "Reproductive and developmental toxicity studies of manufactured nanomaterials", *Reprod. Toxicol.*, **30**(3), 343-352. https://doi.org/10.1016/j.reprotox.2010.06.002.

Fan, Y.O., Zhang, Y.H., Zhang, X.P., Liu, B., Ma, Y.X. and Jin, Y.H. (2006), "Comparative study of nanosized and microsized silicon dioxide on spermatogenesis function of male rats", *J. Hyg. Res.*, **35**(3), 549.

Farshad, A., Khalili, B. and Fazeli, P. (2009), "The effect of different concentration of glycerol and DMSO on viability of markhoz goat spermatozoa during different freezing temperatures steps", *Pakistan J. Biol. Sci.* **12**(3), 239-245. https://doi.org/10.3923/pjbs.2009.239.245.

Farzinpour, A. and Chobdarian, F. (2012), "Effects of silver nanoparticles administration on reproductive performance of Japanese quail", *Avian Biol. Res.*, **5**(3), 164-165.

Farzinpour, A., Karashi, N. (2012), "The effects of nanosilver on egg quality traits in laying", *Appl Nanosci.*, **3**(2), 95-99. https://doi.org/10.1007/s13204-012-0097-5.

Fathi, N., Hoseinipanah, S.M., Alizadeh, Z., Assari, M.J., Moghimbeigi, A., Mortazavi, M. and Bahmanzadeh, M. (2019), "The effect of silver nanoparticles on the reproductive system of adult male rats: A morphological, histological and DNA integrity study", *Adv. Clin. Exp. Med.*, **28**(3), 299-305. https://doi.org/10.17219/acem/81607.

Foldbjerg, R., Olesen, P., Hougaard, M., Dang, D.A., Hoffmann, H.J. and Autrup, H. (2009), "PVP-coated silver nanoparticles and silver ions induce reactive oxygen species, apoptosis and necrosis in THP-1 monocytes", *Toxicol. Letts.*, **190**(2), 156-162. https://doi.org/10.1016/j.toxlet.2009.07.009.

Hussain, S.M., Hess, K.L., Gearhart, J.M., Geiss, K.T. and Schlager, J.J. (2005), "In vitro toxicity of nanoparticles in BRL 3A rat liver cells", *Toxicol. In Vitro.* **19**(7), 975-983. https://doi.org/10.1016/j.tiv.2005.06.034.

Kasai, K., Izumo, A., Inaba, T. and Sawad, T. (2000), "Assessment of fresh and stored duck spermatozoa quality via in vitro sperm-egg interaction assay", *Theriogenology*, **54**(2), 283-290. https://doi.org/10.1016/S0093-691X(00)00348-4.

Kim, J.S., Kuk, E., Yu, K.N., Kim, J.H., Park, S.J., Lee, H.J. and Kim, Y.K. (2007), "Antimicrobial effects of silver nanoparticles", *Nanomed. Nanotechnol. Biol. Med.*, **3**(1), 95-101. https://doi.org/10.1016/j.nano.2006.12.001.

Kwon, J.T., Hwang, S.K., Jin, H., Kim, D.S., Minai-Tehrani, A., Yoon, H.J. and Yoon, B.I. (2008), "Body distribution of inhaled fluorescent magnetic nanoparticles in the mice", *J. Occup. Health*, **50**(1), 1-6. https://doi.org/10.1539/joh.50.1.

Lankveld, D.P., Oomen, A.G., Krystek, P., Neigh, A., Troost-de, J.A., Noorlander, C.W., Van Eijkeren, J.C., Geertsma, R.E. and De Jong, W.H. (2010), "The kinetics of the tissue distribution of silver nanoparticles of different sizes", *Biomaterials*, **31**(32), 8350-8361. https://doi.org/10.1016/j.biomaterials.2010.07.045.

Makhluf, S.B.D., Arnon, R., Patra, C.R., Mukhopadhyay, D., Gedanken, A., Mukherjee, P. and Breitbart, H. (2008), "Labeling of sperm cells via the spontaneous penetration of Eu3+ ions as nanoparticles complexed with PVA or PVP", *J. Phys. Chem. C*, **112**(33), 12801-12807. https://doi.org/10.1021/jp804012b.

Moretti, E., Terzuoli, G., Renieri, T., Iacoponi, F., Castellini, C., Giordano, C. and Collodel, G. (2013), "In vitro effect of gold and silver nanoparticles on human spermatozoa", *Andrologia*, **45**(6), 392-396. https://doi.org/10.1111/and.12028.

Oberdörster, G., Oberdörster, E. and Oberdörster, J. (2005), "Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles", *Environ. Health Perspect*., **113**(7), 823-839. https://doi.org/10.1289/ehp.7339.

Rezaei, A., Farzinpour, A., Vaziry, A. and Jalili, A. (2018), "Effects of silver nanoparticles on hematological parameters and hepatorenal functions in laying Japanese quails", *Biol. Trace Elem Res*., **185**(2), 475-485. https://doi.org/10.1007/s12011-018-1267-4.

Santiago-Moreno, J., Castaño, C., Coloma, M.A., Gómez-Brunet, A., Toledano-Díaz, A., López-Sebastián, A. and Campo, J.L. (2009), "Use of the hypo-osmotic swelling test and aniline blue staining to improve the evaluation of seasonal sperm variation in native Spanish free-range poultry", *Poultry Sci*., **88**(12), 2661-2669. https://doi.org/10.3382/ps.2008-00542.

Silver, S., Phung, L.T. and Silver, G. (2006), "Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds", *J. Ind. Microbiol. Biotechnol*., **33**(7), 627-634. https://doi.org/10.1007/s10295-006-0139-7.

Takeda, K., Suzuki, K.I., Ishihara, A., Kubo-Irie, M., Fujimoto, R., Tabata, M. and Sugamata, M. (2009), "Nanoparticles transferred from pregnant mice to their offspring can damage the genital and cranial nerve systems", *J. Health Sci*., **55**(1), 95-102. https://doi.org/10.1248/jhs.55.95.

Taylor, U., Klein, S., Petersen, S., Kues, W., Barcikowski, S. and Rath, D. (2010), "Nonendosomal cellular uptake of ligand-free, positively charged gold nanoparticles", *Cytometry A*, **77**(5), 439-446. https://doi.org/10.1002/cyto.a.20846.

Win, M.W., Tatemoto, H., Ashizawa, K. and Nakada, T. (2006), "Effect of diethylstilbestrol administration on sperm penetration into the inner perivitelline layer of Japanese quail, Coturnix japonica", *J. Poultry. Sci*., **43**(1), 67-74. https://doi.org/10.2141/jpsa.43.67.

Wiwanitkit, V., Sereemaspun, A. and Rojanathanes, R. (2009), "Effect of gold nanoparticles on spermatozoa: The first world report", *Fertil. Steril*., **91**(1), e7-e8. https://doi.org/10.1016/j.fertnstert.2007.08.021.

Yoshida, S., Hiyoshi, K., Ichinose, T., Takano, H., Oshio, S., Sugawara, I. and Shibamoto, T. (2009), "Effect of nanoparticles on the male reproductive system of mice", *Int. J. Androl*., **32**(4), 337-342. https://doi.org/10.1111/j.1365-2605.2007.00865.x.

Zakhidov, S.T., Pavlyuchenkova, S.M., Marshak, T.L., Rudoy, V.M., Dement'eva, O.V., Zelenina, I.A. and Skuridin, S.G. (2012), "Effect of gold nanoparticles on mouse spermatogenesis", *Biol. Bull*., **39**(3), 229-236. https://doi.org/10.1134/S1062359012030156.

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