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Toxicity of silver nanoparticles on Endometrial Receptivity in Female Mice

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Abstract

Nanoparticles (NPs) have many toxic effects on fertility and can prevent successful implantation by affecting the maternal uterine tissue. Herein, by deploying thirty female NMRI mice, the effect of silver nanoparticles on the endometrium and implantation has been investigated. Using spherical silver nanoparticles of a diameter of 18-30 nm at doses of 2 and 4 mg/kg, mice in both groups were treated. Then, female mice mated with male mice. Endometrial tissue was extracted 4.5 days later. On the fourth day of pregnancy, the mice were anesthetized and blood samples were taken from the heart; furthermore, endometrial tissue was isolated and used for molecular tests, ICP, and examination of pinopods. The results revealed that the levels of *IL6* and *IL1 β* and the accumulation of nanoparticles in endometrial tissue in the group receiving nanoparticles at a dose of 4 mg/kg had a major increase relative to the other two groups ($p < 0.05$); group receiving a dose of 4 mg/kg, exhibited a decrease in pinopods and microvillus compared to the other two groups. According to the results, NPs can reach the endometrium, suggesting that caution should be exercised due to serious exposure to nanoparticles throughout pregnancy.

Keywords: Endometrium, Implantation, Pregnancy, Silver nanoparticles

Introduction

The effect of nanotechnology products on public health has been examined in both in-vitro and in-vivo during the last decade; exposure of pregnant women to nanoparticles (NPs) is a major concern (Ajdary et al. 2018; Sharma et al. 2015). Due to the changes and sensitivity of a variety of types of tissues to environmental pollution, exposure to the nanoparticles throughout pregnancy has been considered (Huang et al. 2015). The endometrium is a key organ for implantation in mammals and its condition is an important aspect that influences the preservation of the embryo and the success of the implantation. Implantation is influenced by two key factors, one is the quality of the fetus, and another is the interaction between the fetus and the endometrium. Therefore, any tissue damage to the endometrium causes the failure of implantation (Ajdary et al. 2020; Huang et al. 2015). Some investigations have assessed the toxicity of nanoparticles on maternal and fetal placental tissues after intravenous injection (Rattanapinyopituk et al. 2013). However, only a few studies have examined the effect of nanoparticles on the organs involved in reproduction (Negahdary et al. 2015). As a whole, the data obtained indicate that exposure of uterus and fetus to nanoparticles is a concern and may instigate a variety of diseases, both directly and indirectly.

There are unique mechanical, thermal, magnetic, and optical properties exhibited by nanoparticles that have been widely exploited for biomedical research and in many industrial sectors (Zhang et al. 2008). Herein, we decided to investigate the intravenous dispersal of silver nanoparticles on endometrial tissue in pregnant mice. There are two major reasons for selecting

the silver nanoparticles; first, they are extensively deployed in consumer products, namely in deodorant sprays, air conditioners, cosmetics, and pesticides (Vance et al. 2015). Importantly, silver nanoparticles have been exploited as a model that can be easily produced and identified using various techniques to monitor their distribution in the reproductive system. Despite the usage of silver nanoparticles, there are worries about their biological effects on the ecosystem and human health; thus, determination of their toxicity should be of paramount importance. Like many environmental chemicals that have harmful effects on fetal development, nanoparticles can damage the liver (Bartneck et al. 2012), kidney (Lin et al. 2008), and reproductive system (Bai et al. 2010); the female reproductive system is significantly more fragile. Reproductive disorders inevitably lead to abnormal fetal development (Tsuchiya et al. 1996), and the advent and pervasive usage of nanoparticles is a new and emerging threat to the reproductive system. In view of their importance, the toxicity of nanoparticles has been examined in various animal models (Tsuchiya et al. 1996). In an investigation by Yamashita et al., injection of a dosage of 0.8 mg SiO₂ and TiO₂ nanoparticles intravenously in pregnant mice reduced the uterine weight of mice and raised the rate of their fetal reabsorption (Yamashita et al. 2011). Another investigation conducted by Tolaymat et al. showed that nanoparticles, like other toxic chemicals, may have an adverse effect on the reproductive system.

The presence of a large number of nanoproducts in the universe around us can release NPs into the environment, exposing animals and humans to these substances. Exceeding the toxicity threshold dose during embryo implantation can be the source of various events such as abortion, non-implantation, endometrial tissue destruction, and even damage to fetal development. Therefore, it is necessary to be aware of the possible adverse effects of nanoparticles on the embryo implantation process. Recently, many studies have been performed on different stages of

pregnancy, but the effect of nanoparticle toxicity on the endometrium is very rare. The results of Li et al.'s study in 2010 showed that the rate of implanted embryos in mice poisoned with silver nanoparticles was significantly reduced compared to the control group and the embryos that were implanted did not have a successful evolution. Silver nanoparticles caused damage to the trophoctoderm in blastocysts significantly increase apoptosis (Li et al. 2010), which occurred in De et al.'s study on the toxicity of cadmium nanoparticles in 1993 (De et al. 1993b).

Silver nanoparticles are clusters of silver atoms with a size of 1-100 nm (Tolaymat et al. 2010). They are used in various fields because of their unique properties and antibacterial activity. Not surprisingly, they can be consumed right away through water, cosmetics, food, and drug distribution appliances (Rai et al. 2009). Oxidative damage is one of the biggest concerns for the use of nanoparticles; likewise silver nanoparticles, oxidative damages cause stress by binding to membrane structures, and proteins or DNA to generate reactive oxygen species, inflammation, and finally, cell death or apoptosis (Sivolella et al. 2014). As shown in a study conducted by Park et al., many medical devices release silver ions into the bloodstream, and their accumulation in various tissues causes toxicity in various organs, leading to cell death in a high dose-doctored group (1.00 mg/kg) (Park et al. 2010). In recent years, there have been accounts about the toxic effects of silver nanoparticles on the ovaries, which affect ovulation by activating the cytokines and chemokines pathways and causing inflammation (Ghorbanzadeh et al. 2011). While silver nanoparticles are used widely in nanotechnology and nanomedicine, but their harmful effects on the endometrium have not yet been thoroughly investigated. Consequently, this investigation was undertaken to assess the toxicity of silver nanoparticles on endometrial receptivity in female mice.

Materials and Methods

Animals

In this study, 30 female mice with a weight range of 30-50 g were kept in standard cages in the Animal House of Iran University of Medical Sciences. Prior to the experiments, the mice were fed a normal diet and water to adapt to the environment and to develop physiological adaptation. During the study, the animals were kept under comparable conditions at a temperature of 22 ± 2 °C with 12-hour light and 12-hour darkness and freely accessing food and water. All studies were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC.1397.1211), Tehran, Iran.

Chemicals

The chemicals used in this study were procured as follows: TRIzol reagent (Sigma, Pool, UK), SYBR Green PCR Master Mix (Invitrogen, Paisley, UK), AgNPs (Neutrino Chemical Company, Tehran, Iran), ELISA kit (Abcam 108666, Cambridge, MA, USA), cDNA Synthesis Kit (Invitrogen, Paisley, UK), and Glutaraldehyde (Sigma, German).

Nanoparticle synthesis method

A total of 30 mL of sterile distilled water was placed in a sterile Erlenmeyer flask inside the beaker containing ice. A total of 67 μ L of 2 mM sodium borohydride solution was added and 1 mL of 2 mM AgNO_3 solution was added dropwise to obtain a yellow solution. Erlenmeyer flask was then removed from the ice and its contents added dropwise to Erlenmeyer containing 20 mL

of 2 mM trisodium citrate solution placed on a shaker at 150 rpm. The final solution was divided into 1.5 mL microtubes and centrifuged at 14000 g for 15 minutes. To remove contaminants and excess reaction materials, the nanoparticles were washed three times using sterile distilled water. The final precipitate was dispersed in 10 mL of sterile distilled water.

Properties of nanoparticles

Nanoparticles had purity properties: ≥ 99.9 wt%, specific surface area : >30 m²/g (SSA), actual particle size (APS): 30 nm, density: 10.5 g/cm³, volume density: 0.5 g/cm³, appearance: spherical, color: gray, and preparative methods: laser-induced chemical vapor deposition and were used in the study.

Experimental protocol

Before mating and administering nanoparticles, the vaginal smear method was used to ensure the regularity of animal cycles. Mice were divided into three groups (n=10) and they received doses of 2 mg/kg and 4 mg/kg (Austin et al. 2012; Docea et al. 2020).

A 2 mg/kg dosage of intravenously injection in mice tail silver nanoparticles (30 nm) was given to group I on a daily basis for two weeks. A 4 mg/kg dosage of silver nanoparticles intravenously injection in mice tail was given group II each day for two weeks and the control group received saline intravenously on a daily basis for two weeks; then, female mice mated with male mice. We set the day of vaginal plaque visibility as GD 0.5. Endometrial tissue was extracted 4.5 days later at the time of the implantation window.

Tissue collection

Endometrial samples were divided into three parts. One sample was placed in glutaraldehyde, which was used to study the tissue morphology by Transmission Electron Microscopy (TEM),

and another one was placed at -80 degrees for molecular analysis; also, the tissue sample was used to study the accumulation of nanoparticles.

Investigation of nanoparticle accumulation in endometrial tissue

The data were processed to calculate the distribution and concentration of the particles were determined using ICP-MS (Inductively Coupled Plasma-Mass Spectrometry System Yokogawa Analytical Systems Inc., Tokyo).

TEM analysis

Using TEM (Hitachi H-300; Hitachi Ltd, Tokyo, Japan) and with the help of these endometrial tissue samples, we observed the endometrium's morphological features.

Hormone assay

Separation of blood serum from the gathered blood was done via centrifugation (1500 g for 10 minutes. Then, serum samples were kept in a -20 °C deep freezer. Using enzyme-linked immunosorbent assay (Abcam 108666, Cambridge, MA, USA), measurement of blood estradiol concentration was performed (Smeriglio and Wilcox 1999).

Extraction of RNA and real-time quantitative reverse- transcription PCR (Q-PCR)

According to the manufacturer's instructions, extraction, and purification of all tissue RNAs were executed by TRIzol reagent (Sigma, Pool, UK); RNA concentration was measured with a Nano Drop ND-100 spectrophotometer. The reverse transcription-polymerase chain reaction (RT-PCR) was completed to monitor the gene expression levels of *IL6*, *IL1 β* . Designing primers were particularly performed among two contiguous exons (Gene Runner program). Table 1 exhibits the sequences utilized in this research. By subtracting the *β -actin* Ct value from the Ct value of the sample ($\Delta\text{Ct} = \text{Ct}_{\text{Sample}} - \text{Ct}_{\text{Reference}}$), normalization of the mRNA levels of these genes (Ct)

was done to that of the reference gene *Actin, beta* (β -actin). Deploying $2^{-\Delta\Delta C_t}$, the relative expression of the target gene to the calibrator was quantified (Hajihosseini et al. 2015).

Table 1. Sequence of primers *IL6* & *IL1 β*

Gene		Sequence (5'->3')	Length	Tm	GC%	Self-complementarity	Self-3' complementarity
<i>IL-6</i>	Forward primer	AGACTTCCATCCAGTTGCCTT	21	59.29	47.62	3.00	0.00
	Reverse primer	TTCCACGATTTCCCAGAGAACA	22	59.63	45.45	5.00	3.00
<i>IL1β</i>	Forward primer	TGCCACCTTTTGACAGTGATGA	22	59.38	45.45	5.00	2.00
	Reverse primer	TTGATGTGCTGCTGCGAGATT	21	60.42	45.45	3.00	2.00
<i>Actin, beta</i> (β -actin)	Forward primer	CAAGATCATTGCTCCTCCTG	20	60.95	47.62	4.00	2.00
	Reverse primer	ATCCACATCTGCTGGAAGG	19	57.3	52.60	6.00	0.00

The enzyme-linked immunosorbent assay (ELISA)

IL-1 β in the tissue homogenates was determined by ELISA utilizing a commercial kit from R&D Systems (Minneapolis, MN). *IL-6* was ascertained by immunoradiometric methods utilizing mercantile kits made by Medgenix SA (Fleurus, Belgium).

Statistical analysis

The usual distribution of the data was assessed using the Kolmogorov-Smirnov test. The Mean $\pm$ SEM was used for presenting all data. One-way analysis of variance (ANOVA), accompanied by Tukey's post hoc test, was employed for determining the statistical importance

of disparities between the groups. All data were analyzed with the software GraphPad Prism version 8.0, and P-values less than 0.05 were taken into account statistically important.

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221 **Results**

222 The morphology and size of the synthesized AgNPs were determined by using transmission
223 electron microscopy (TEM). The images clearly showed that the average size of spherical shaped
224 particles to be ~18-30 nm (A Figure 1). According to the XRD pattern, the Miller indices at the
225 levels (200), (220), (311) corresponded to the angles of 46.255°, 64.51°, and 77.011°,
226 respectively, which affirmed the presence of silver nanoparticles. The DLS data of AgNPs show
227 the size of 30 nm and the zeta potential of -18 Mv (C, D Figure 1).

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Figure 1. Physicochemical properties of AgNPs; (A) TEM image of AgNPs; (B) XRD image; (C) particle size by DLS; (D) Zeta potential of AgNPs

Effects of AgNPs on serum reproductive hormone level

As depicted in Figure 2, the mean of estradiol serum levels in the 4 mg/kg subjects was significantly less than in animals assigned to both groups ($P<0.05$).

Figure 2. AgNPs' effects in serum levels of the reproductive hormone in NMRI mice. Expression of data is as the Mean $\pm$ SEM; *Significant decrease of the hormone level in the 4 mg/kg group compared with the control groups ($P<0.05$).

Endometrial morphology

Figure 3 presents the TEM images of the ultrastructure of the endometrium for the mice receiving 14-day AgNPs. Figure 3 A shows that the endometrium ultrastructure of the mice in the control group obviously did not indicate apparent abnormality in the epithelial morphology, and structure and the junction of cells can be detected as usual. Figure 3 B shows the

endometrium ultrastructure of the AgNPs (2 mg/kg) group; mitochondria have been extremely deformed, and a number of cells in the stroma collapsed. Figure 3 C shows the endometrium ultrastructure in the AgNPs (4 mg/kg) group I. In terms of mass, the endometrial cells' structure was nearly normal; the endometrial epithelial cells' microvilli became shorter and thinner.

Figure 3. TEM images of the endometrium ultrastructure for the mice; (A) The control group; (B) The nano-AgNPs group (2 mg/kg); (C) The nano-AgNPs group (4 mg/kg) apparently the injury to the of mice' endometrium produced by the nano-silver is more severe than what triggered by the AgNPs group (2 mg/kg) ($\times 10\mu\text{m}$). P: pinopode, M: microvilli. Scale bar = $1\mu\text{m}$.

ICP-MS analysis

Table 2 summarizes the results of the quantitative distribution of AgNP tissue and blood attained for the 2 mg/kg, 4 mg/kg exposure groups by sp ICP-MS. In endometrium, mass concentration of AgNPs 4 mg/kg was 0.0049 ± 0.0011 mg/kg of tissue. In addition, particles that had a diameter more than the size detection limit (approximately 30 nm) were constantly $\sim 5\%$. In the control group samples, AgNPs were not identified (the mean mass concentration lower than the detection limit). In blood, the mass concentration of AgNPs 4 mg/kg was 0.018 ± 0.001 mg/kg of tissue. In addition, particles that had a diameter more than the size detection limit (~ 30 nm) were constantly $\sim 5.21\%$. In the control group samples, AgNPs were not identified (the mean mass concentration was lower than the detection limit).

265 **Table 2.** Results of the quantitative distribution of endometrium of Ag NPs intended for the 2, 4
266 mg/kg exposure group by sp ICP-MS, as expressed based on a wet weight (n=10).

Groups	Nanoparticle aggregation site	Size mode (nm)	Size mean (nm)	Particle number concentration (#parts/g)	Particle mass concentration (mg/kg)	<size DL & Ionic Ag mass concentration	Total Ag (mg/kg)
2 mg/kg	Endometrium	< limit of detection (LOD)	<LOD	<LOD	<LOD	0.012±0.007 (0.0039-0.0159)	0.012±0.006 (0.005-0.017)
4 mg/kg	Endometrium	30±5 (20-30)	30±10 (25-30)	6.4×10 ⁷ ±1.9×10 ⁷ (5.5×10 ⁷ ±8.6×10 ⁷)	0.0049±0.001 (0.004-0.006)	0.077±0.009 (0.0671-0.0851)	0.082±0.010 (0.071-0.091)
2 mg/kg	Blood	<LOD	<LOD	<LOD	<LOD	0.210±0.004 (0.200-0.222)	0.224±0.021 (0.218-0.230)
4 mg/kg	Blood	30±5.21 (18-30)	30±11.11 (28-30)	8.4×10 ⁸ ±10.2×10 ⁷ (6.4×10 ⁸ ±9.1×10 ⁸)	0.018±0.001 (0.008-0.033)	0.140±0.007 (0.117-0.142)	0.140±0.020 (0.125-0.145)

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268 ***Effects of AgNPs on genes of the female fertility-related pathways***

269 The mRNA levels of *IL-1β* were considerably higher in the group receiving 4 mg/kg compared
270 to the control group and the group receiving 2 mg/kg (*P*<0.05) (Figure 4).
271 Figure 4 shows that the mRNA levels of *IL-6* were appreciably superior in the group receiving 4
272 mg/kg compared with the control groups (*P*<0.01).

Figure 4. *IL-1β* (A) and *IL-6* (B) mRNA expression in endometrium tissues from female mice exposure to AgNPs. Data are the Mean±SEM (n=10 for each group). B-actin was employed as an internal control. * $P < 0.05$ exposure to AgNPs group vs. control groups † exposure to AgNPs group vs. 2 mg/kg.

Confirmation of PCR-real time results by ELISA analysis

The PCR-real time results were confirmed by ELISA analysis of all proteins, including *IL6*, *IL-1β* (Figure 5). *IL6*, *IL-1β* protein concentrations were considerably better in the group receiving 4 mg/kg compared to the normal controls ($p < 0.05$). *IL6*, *IL-1β* protein concentrations in the group receiving 4 mg/kg were significantly higher compared to the group receiving 2 mg/kg ($p < 0.05$).

Figure 5. *IL-1β*, *IL6* protein. (A) Concentration of *IL-1β* protein (ng/mg total proteins); (B) concentration of *IL6* protein (ng/mg total proteins) being evaluated by ELISA in endometrial tissue protein extracts. Data are the Mean $\pm$ SEM (n=10 for each group). * P <0.05 exposure to AgNPs group vs. control groups † exposure to AgNPs group vs.2 mg/kg.

Discussion

The results showed that the amount of *IL6-IL1 β* and the accumulation of nanoparticles in endometrial tissue in the group receiving nanoparticles at a dose of 4 mg/kg had a noteworthy rise relative to the other two groups ($p < 0.05$); pinopods and microvillers in the 4 mg/kg group decreased compared to the other two groups. Immunofluorescence studies have shown that type 1 interleukin receptor is present in the endometrial epithelium and has a significant increase before implantation. Molecular factors affecting embryo implantation and how these factors are identified can be key factor in the reproductive control mechanism. However, successful implantation depends on a well-proportioned interaction between the blastocyst and the embryo. It is also, to some extent, controlled by paracrine cytokines. In fact, effective factors in implantation include two types of interleukins α and β , and their receptor, and receptor antagonist. Interleukin receptors also include two types of receptors: I and II (Horuk and McCUBREY 1989). Successful implantation can be predicted if the amount of interleukin in IVF culture media mediums is high. Studies indicate that the level of interleukin in pregnant mice is increased before implantation, and in the fourth and fifth days of pregnancy, its amount reaches a maximum; therefore, by binding to interleukin receptors and blocking it, it can have an effect on implantation (De et al. 1993a). Research shows that growth factors and cytokines both play a major role in the interaction between fetus and mother so that in mice, the growth factors can replace estrogen in endometrial proliferation (Simon et al. 1994). To investigate the reaction of the interleukin receptor and its receptor antagonist, further research has been conducted. Reproduction of the mutated mice lacking interleukin receptors has been another maneuver executed by researchers in this field (McIntyre et al. 1991).

Silver nanoparticles are a group of nanoparticles that are extensively deployed in commercial medicine and in manufacturing various products such as antiseptic sprays and antimicrobial bandages due to their antimicrobial properties. While these materials are used widely, there is still a scarcity of information about their effect on health. Besides, the results obtained from various studies are different due to the presence of large variations of sizes, coatings, or particle shapes. Although the silver ions released from silver nanoparticles are undoubtedly toxic, there is still no conclusive evidence as to whether metallic silver nanoparticles themselves have particle-specific toxicity. Metallic silver is easily oxidized in acidic liquid solutions exposed to air to generate toxic silver ions; in fact, toxicity depends on the concentration of silver ions released under aerobic conditions (Whitman et al. 2008). As shown in the studies, even a low dose of the silver nanoparticles have severe effects on the human macrophages (Haase et al. 2011); they can cause changes in cellular DNA due to the formation of free radicals. Oxidative stress causes inflammation in the tissue by forming free radicals. Because silver nanoparticles have a larger surface area, they are more chemically active and become more ionized; they can bind to various tissues and have toxic and destructive effects due to the production of oxygen free radicals that eventually causes cell death (Cramer et al. 2014).

The importance of nanoparticle exposure during pregnancy has recently been considered (Figueroa-Espada et al. 2020; Li et al. 2012). However, there are still shortcomings in this regard, which have recently been identified by a comprehensive study on this subject (Hougaard et al. 2015). According to the results of our present study, exposure to silver nanoparticles causes the buildup of nanoparticles in endometrial tissue and induces an unfavorable pregnancy outcome, the results of which were dose-dependent.

In our study, the presence of silver nanoparticles in endometrial tissue was determined by ICP, and the change in pinopods was established by TEM. ICP-MS analysis showed that the amount of silver nanoparticles in the endometrium was 2 mg/kg (0.012 ± 0.006) in the group and 4 mg/kg (0.082 ± 0.010) in the group indicating that the nanoparticles can pass through the uterus and can affect implantation. In fact, secondary nanoparticles are generated after the interaction of the released silver ions with the sulfur-bearing group of proteins and/or the selenium or chloride present (Juling et al. 2016). The size and chemistry of nanoparticles and the dose are effective for passing through the reproductive system (Blum et al. 2012).

Yu et al. showed that high doses of silver nanoparticles, especially ≥ 100 mg/kg/day, drastically reduced the embryo-fetal development in the mouse embryo, which confirms the severe toxicity of silver nanoparticles (Yu et al. 2014). Nanoparticles can induce harmful effects on the reproductive system of mice and fetal development *in vitro* and *in vivo*. As shown in studies of mice, exposure to nanoparticles causes nanoparticles to settle in the fetus via the placenta; besides, inflammation caused by nanoparticles is the most important factor in embryonic, neuronal and reproductive toxicity (Sun et al. 2013). The biodistribution of many nanoparticles is size-dependent; besides, smaller nanoparticles can better communicate with the nanoparticle functional units and exert their effects. Lytvynenko et al. have shown that administration of AgNPs (2 mg/kg and 4 mg/kg) results in inhibition of oocyte meiotic maturation, upsurges the number of apoptotic cells, and necrotic follicular cells surrounding oocytes in mice (Lytvynenko et al. 2017).

Ferdous et al. showed that dispensation of AgNPs distributes them to tissues, organs, and causes histological damage; also, structural changes depend on the type of tissue, the injection dose, and the response time. Additionally, there was apoptosis and reduction in the embryo-fetal

development were observed at high doses (Ferdous and Nemmar 2020). However, the size of nanoparticles alone does not necessarily determine the toxicity of nanoparticles. Besides the size, shape, and potential accumulation of nanoparticles can also be effective in regulating their toxicity (Tian et al. 2009). Zhang et al. have examined the effect of 8 nm spherical AgNPs extracted from *Bacillus cereus* on pregnant mice by administering them intravenously at a dose of 1 mg/kg for 6.5 days. On days 13.5, 15.5, and 17.5 after pregnancy, the mice were anesthetized, and the embryo and placenta were extracted. The status of ovarian meiosis, DNA methylation, and embryonic disorders was assessed. Exposure to AgNPs disrupts the *Ascl2*, *Snrpn*, *Kcnq1ot1*, *Peg3*, *Zac1*, *H19*, *Igf2r*, and *Ig* genes. DNA methylation studies disclosed that exposure to AgNPs appreciably changed the methylation levels of various *Zac1* methylated regions. The results point out that silver nanoparticles degrade the epigenetic status of the placenta and fetus (Zhang et al. 2015).

In a 2012 study, Austin et al. examined the toxic effects of Ag NPs on pregnant mice and fetuses deploying Ag NPs of diameter 50 nm at 35 and 66 mg, which were injected intravenously on days 7, 8, and 9 of pregnancy, and on the tenth day they were anesthetized, and tissue samples were collected. The results showed that the accumulation of nanoparticles in the tissues of the liver, spleen, lung, and tail (injection site) of visceral yolk sac and endometrium was higher compared to other organs. In summary, the results showed that Ag NPs were distributed in most maternal organs, extra-embryonic tissues and embryos, but did not accumulate significantly in embryos (Austin et al. 2012), which is consistent with the results of the present findings. In this study, accumulation of nanoparticles has been observed in endometrial tissue, but in our investigation, the main purpose of embryo implantation was the factor involved in this pathway which was affected due to the toxicity of nanoparticles.

Conclusion

In conclusion, this study has shown that AgNPs cause damage to the mother's major organs and affect the implantation due to the discharge of inflammatory factors from the uterus. These results indicate that NPs can reach the endometrium suggesting that caution should be exercised due to acute exposure to nanoparticles through pregnancy, especially in jobs that have exposure to silver nanoparticles. This is especially important for pregnancy outcomes, as early exposure, when pregnancy status is not diagnosed) is the most dangerous.

Author Contributions

M.A. and S.E. contributed to the writing of the original draft and contributed to the conceptualization of the work. V.P. Performed the TEM technique. F.K. and R.S.V. conceptualization, writing-review, and editing.

Declaration of Conflicting Interests

The author (s) declared no potential conflicts of interest with respect to the research, authorship, and /or publication of this article.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Institutional Review Board Statement

The study was approved by the Ethics Committee for Animal Experimentation of the Iran University of Medical Sciences.

Informed Consent Statement

Not applicable.

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