PERSPECTIVE STUDY OF THE AgNPs EFFECT ON THE MATERNAL AND EMBRYONIC DEVELOPMENT IN ALBINO RATS

I. H. Ali L. A.Salih H. A. Al-jezani
Assist. Lecturer Assist. Professor Assist. lecturer
Dept. Bio. Coll. Sci. University of Baghdad Dept. Denis. AL- Yarmouc university
Email: esrahassan954@yahoo.com

ABSTRACT

This study was aimed to displayed effect of this nanoparticles on pregnant mother and embryos. All females administration of AgNPs suspension orally during the gestational period (for 21day) in two doses low 2mg and high dose 20 mg /Kg body weight and the control group received D.W only. The pregnant females (60 females) include the control group and the treated group was subdivided in to two groups, pre and post implantation and all the mothers weighted along the study. The embryos and their brains after retrieved weighted and the crow-rump length (CRL) measurement also. The results showed that the active form of Ag can be transport the placental barrier and blood brain barrier (BBB). This nanoparticles showed adverse effect and produced decreased in mothers weights in low dose 2mg/Kg/ B.Wt and higher dose 20mg/Kg/ B.Wt. Weights of embryos were lower clearly after exposure to AgNPs compare to control group. On the other hand, the weights of embryo's brain were decreased compare of control group in both doses. The CRL of embryos lowered after exposure to AgNPs in treatment groups when compare to control group.

Keywords: pregnant females, CRL, brain, gestational period

مجلة العلوم الزراعية العراقية -2019 :50(6):1605-1612 علي وآخرون

دراسة استطلاعية لتأثير دقائق الفضة المتناهية الصغر على الامهات والتطور الجنيني في الجرذان البيض

أسراء حسن علي لينا عبد المطلب صالح حسن علي الجيزاني
مدرس مساعد استاذ مساعد مدرس مساعد
كلية العلوم/ جامعة بغداد/ قسم علوم الحياة كلية اليرموك /قسم طب الاسنان

المستخلص

تهدف الدراسة الى معرفة تأثير الدقائق النانوية على الامهات الحوامل والاجنة. جميع الاناث جرعت بعالق دقائق الفضة النانوية فمويأ خلال فترة الحمل(البالغه21 يوما) وعلى جرعتين منخفضة 2 ملغم وجرعة عالية 20 ملغم / كم / من وزن الجسم اضافة الى مجموعه السيطرة التي جرعت بالماء المقطر . كل الاناث الحوامل (60 انثى) قد تم تقسيمها الى مجموعة السيطرة اضافتا الى مجموعتين ثانويتين ضمن كل جرعه، مجموعة قبل الانغراز ومجموعة ما بعد الانغراز وكل الامهات قد تم وزنها طول فترة الدراسة، الاجنة وادمغة الاجنة بعد ان تم استخراجها قد وزنت ايضأ وكذلك الطول من التاج الى الارداف للأجنة CRL قد تم قياسها ايضأ. أظهرت النتائج ان الشكل الفعال لدقائق الفضة يستطيع ان ينتقل عبر حاجز المشيمة وحاجز الدماغ الدموي. أظهرت هذه الدقائق النانوية تأثيرا مؤذي من خلال تقليل اوزان الامهات في الجرعة المنخفضة 2 ملغم وكذلك في الجرعة العالية 20 ملغم. اظهرت النتائج ان اوزان الاجنة قد انخفضت بشكل واضح بعد التعرض لدقائق الفضة مقارنتأ مع مجموعة السيطرة. وايضا اوزان ادمغة الاجنة والطول من التاج الى الارداف قد انخفض بعد التعرض لدقائق الفضة النانوية مقارنتا مع مجموعة السيطرة وفي كلا الجرعتين.

الكلمات المفتاحية :اناث حوامل، الطول من التاج الى الارداف، الدماغ، فترة الحمل

***Received:17/3/2019, Accepted:7/7/2019**

INTRODUCTION

Nanotechnology has commonly developed in the last decades as new methods for nanoparticles production and new nanoparticle properties have been discovered **(**10). Nanotechnology used to treatment of material on atomic and molecular form at 1-100 nm (4). Different nanomaterials are being used, but the most common is silver nanoparticles (AgNPs) (16). Humans can be interacting with these particles in different ways, such as oral administration, inhalation, ingestion, injection or skin contact. Silver nanoparticles (AgNPs) are largely used for many properties in various areas such as cosmetics, toothpaste, textiles, and water purification systems and in the medical field (20). AgNPs are getting more and more interest in medical research fields due to their antimicrobial antifungal properties (13, 14). They can be used in many medical applications such as diagnosis of disease, treatment, drug delivery and medical device coating. The female culture is mainly vulnerable and deserves special attention because toxicity in this group to may affects both female reproductively and fetal development. Mouse, rats and other animals' models have their own unique features and studies using these models to examine the potential toxicity of different nanoparticles on these animals. Several nanoparticles exhibit harmful effects on female reproductive system as well as fetal development, and these adverse effects are related to nanoparticle composition, surface modification, dose, exposure route and animal species (23). The nanoparticles can be reach by systemic route and they can transport to the brain through the Blood-Brain Barrier (BBB), following the opening of the tight junctions by hyper osmotic barrier and consequently cause damage in brain by some induction of oxidative stress or inflammatory responses (12, 1). The BBB is composed of a single layer of endothelial cells that are joined by tight junctions and surrounding by astrocytes and cover the surface area of the cerebrovascular capillaries, thus separating circulating blood from extracellular fluid of the central nervous system (CNS) as (7). The BBB functions mainly as a protective barrier for the brain for preventing transition of various elements, including hormones, neurotransmitters neurotoxins or nanoparticles from the blood stream into the CNS (2). Hong *et al*. (2014) reversed standard reproductive/ developmental toxicity endpoints e.g. pre- and post-implantation loss, number of live and dead pups, external abnormalities in pups found no effects. Some of these published studies suggest a potential for developmental effects following in uteri AgNPs exposure; though, further study of post-exposure silver tissue distribution and potential effects on embryonic development, preferably using a consistent experimental design, is more needed.

MATERIALS AND METHODS

Preparation of silver nanoparticles (AgNPs) suspension

AgNPs were prepare in this study as a suspension, it was purchased as grey black solid powder (purity 99.9%, apparent density: 0.97g /ml, tap density: 2.16 g/ml and CAS NO.: 7440-22- 4) with an average diameter between (40-60) nm in diameter by using SPM device. AgNPs were prepared at a two concentrations, low concentration (2mg/kg) of body weight and high concentration (20mg/kg) of body weight according to (9). The AgNPs stock solution was prepared by suspending the calculated weight of AgNPs powder in a certain volume of deionized distilled water in a sterile glass universal tube. The suspension was exposed to the ultrasonication technique by using ultrasonic water bath for 2-3h in dark condition and under biological safety Figure (1).

Fig. 1. AgNPs suspension

Characterization of AgNPs

The spherical shape of silver nanoparticles was characterized by using scanning probe microscope (SPM) by granularity accumulation distribution report, the average

diameter in sample contain about 260 grain was (40-60 nm) as the following report in (Figure 2).

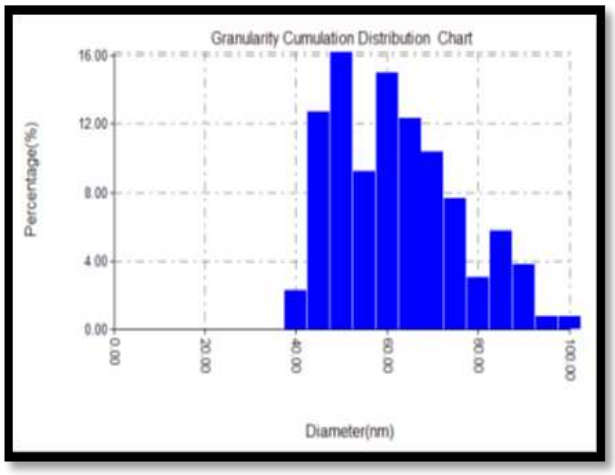

Fig. 2. Granularity cumulating distribution chart

Administration of AgNPs to the female rats

In present study, the AgNPs suspension was given to the treated groups orally (gavage route) in a volume of 2 ml of AgNPs daily during pregnancy period by using polyethylene orogastric tubes (feeding tube) connected to a syringe in appropriate size. The dosage was in milligram per kilogram body weight (mg/Kg/B.wt). D.D.W was used as the vehicle for AgNPs preparation, while the control group was given D.D.W only.

Embryos examination

The pregnant female albino rats were fully anesthetized by diethyl ether for several minutes. The female weighted before and after administration, then the pregnant females were killed to remove the embryos in different gestation days (GD). Abdominal midline incision was performed, the two uterine horns were removed, the embryos were extracted from the placental sacs, and the extra-embryonic membranes were then removed, rinsed in normal saline. All the embryos weighted and measure the CRL then, the embryo was examined under the dissecting microscope to remove the embryos brain (Figure 3& 4).

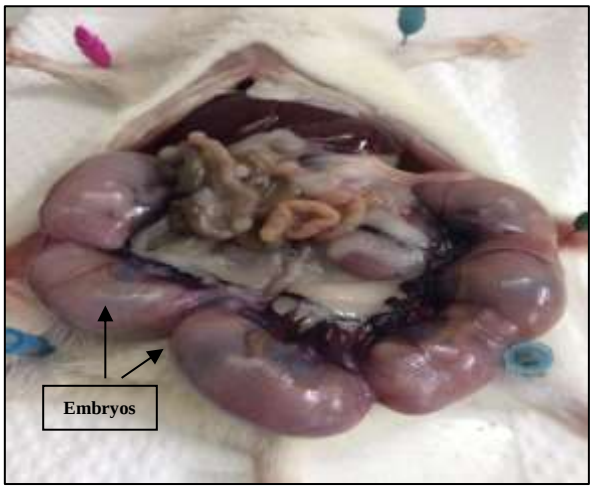

Fig. 3. Embryos in uterus horns, the black arrow shows embryo inside embryonic sac in uterus

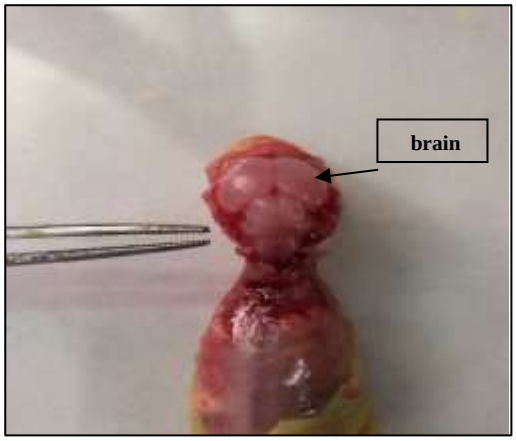

Fig. 4. The embryos brain

Morphological measurements of the embryos

Crown-Rump length (C-R Length mm)

The C-R length is the largest length of the ventrally curved embryo-based on this length. The CRL was examined for all retried embryos (Figure 5).

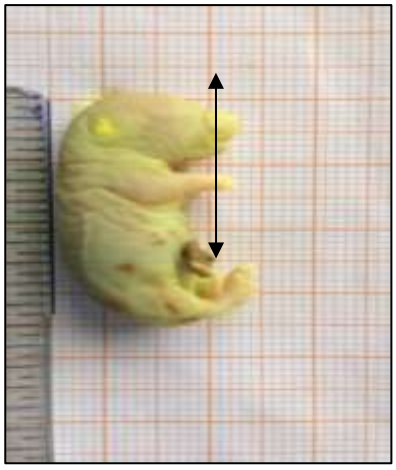

Fig. 5. CRL of rat embryo

Statistical analysis

The Statistical Analysis System- SAS (22) program was used for data analysis. One way ANOVA and lest significant difference LSD were used to assess the significant difference between means and Chi-Square: $\chi 2$ test was used to compare the difference between percentages in this study

RESULTS AND DISCUSSION

In this study, we used many parameters to detect the effect exposure of AgNPs on mother and embryos during the pregnancy. The results showed there is no significant differences ($P \geq 0.05$) in mothers weights in pre and post implantation after exposure to AgNPs in both doses; while, the results showed decreased in mothers weights in low dose 2mg/Kg/B.Wt and higher dose 20mg/Kg/B.Wt of AgNPs in pre and post implantation was clear compare with control group (Table 1)

Table 1. Effect of AgNPs on the body weights of mothers (g): pre and post implantation (Mean ± SE) in the albino rats

The Groups	Implantation		LSD value
	Pre	Post	
G1: Control	213.71 ± 9.52 g a	213.71 ± 9.52 g a	---
G2 :2mg AgNPs	A 162.07 ± 13.78 g b	A 172.41 ± 11.97g a	12.69 NS
G3: 20 mg AgNPs	A 165.31 ± 10.66 g b	A 167.01 ± 8.25 g b	9.57 NS
LSD value	17.94 *	15.61 *	---
* (P<0.05), NS: Non-Significant.			

Mean with a different capital letter in a same row significantly different (P≤0.05) mean with a different small letter in the same columns significantly different (P≤0.05)

On the other hand, the results showed a significant decrease ($P \leq 0.05$) in embryos weights after the mothers were exposed to AgNPs in both doses as compared with the control group. The embryos weights were decreased significantly ($P \leq 0.05$) in pre-implantation and in post-implantation group in both doses (Table 2).

Table 2. Effect of AgNPs on embryos weight (g): pre and post implantation (Mean ± SE)

The Groups	Implantation		LSD value
	Pre	Post	
G1: Control	1.54 ± 0.07 g a	1.54 ± 0.07 g a	----
G2:2mg AgNPs	B 1.01 ± 0.04g b	A 1.52 ± 0.08g a	0.473 *
G3:20mg AgNPs	B 0.260 ± 0.02 g c	A 0.976 ± 0.03g b	0.369 *
LSD value	0.309 *	0.462 *	----
* significant differences (P≤0.05)			

Mean with a different capital letter in a same row significantly different (P≤0.05) mean with a different small letter in the same columns significantly different (P≤0.05)

The embryos brains were weighted also after exposure to AgNPs during pregnancy. The result displayed no significant differences ($P \leq 0.05$) in weights of embryos brains in low dose of AgNPs (2mg)(0.037 NS), while, there is a significant different ($P \leq 0.05$) in higher dose (20mg) (0.049 *). In both pre-implanted and post-implanted groups the embryos brains were decreased compared to control group, whereas the higher dose (20mg) showed more effect on the brains weight in pre-implanted group as there is a significant decrease (0.051*) in weights of brains compared to control group (Table 3).

Table 3. Effect of AgNPs on the weights of embryos brains (g): pre and post implantation (Mean ± SE)

The Group	Implantation		LSD value
	Pre	Post	
G1: Control	0.16 ± 0.004g a	0.16 ± 0.004g a	----
G2:2mg AgNPs	A0.15 ± 0.002g a	A 0.13 ± 0.003g a	0.037 NS
G3:20mg AgNPs	B 0.09 ± 0.002g b	A 0.15 ± 0.004g a	0.049 *
LSD value	0.051 *	0.044 NS	---
* (P<0.05), NS: Non-Significant.			

Different letters mean significant difference at (P≤0.05)

Although, the crow- rump length CRL of all embryos was measured, it is considered a bio-indicator of embryonic day during pregnancy in rats. The CRL was detected for all embryos. The present results identified a significant decrease (P≤0.05) in CRL of embryos in both doses compared to control group and the higher dose was more effect significantly (P≤0.05) than the lower dose of AgNPs, furthermore; the higher dose was more effect on the CRL in pre-implanted compare of post-implanted embryos compare to control group (Table 4).

Table 4. Effect of AgNPs on embryos (CRL-mm): pre and post implantation (Mean ± SE)

The Group	Implantation		LSD value
	Pre	Post	
G1: Control	40.05 ± 2.69 a	40.05 ± 2.69 a	----
G2:2mgAgNPs	B 33.17 ± 2.07mm b	A 38.00 ± 2.51mm a	4.091 *
G3:20mgAgNPs	B 14.00 ± 0.65mm c	A 31.26 ± 1.86mm b	7.336 *
LSD value	5.963 *	4.883 *	----
* (P<0.05)differed significantly.			

In present study, we measured the percentage of mothers that given embryos (14 mothers in pre implantation group, 11 mother in post implantation) compare to mothers that not gave complete embryos (not given embryos), (20 mother in pre and 7 in post implantation group). The results showed that the effect of AgNPs on pre implantation embryos and mothers more than the post implantation group in both low and high dose. In pre implantation and post implantation group the higher dose 20 mg/kg was more effect on production of embryos than the low dose 2mg/kg compared with the control group (Table5).

Table 5. Effect of AgNPs on the number and percentage of embryos: in pre and post implantation groups

Pre implantation			Post implantation		Chi-Square: χ^2	
No. of mother with embryos			No. of mother with embryo			
G1: Control	G2 : 2mg	G3: 20mg	G2 : 2mg	G3 : 20mg		
7	9	5	6	5		
(87.50%)	(52.94%)	(29.41%)	(66.67%)	(55.56%)	10.26**	No. of mother
No. of mother without embryos			without embryos			
1	8	12	3	4		----
(12.50%)	(47.06%)	(70.59%)	(33.33%)	(44.44%)	10.26**	
No.= 8	No.= 17	No.= 17	No.= 9	No.= 9	---	
χ^2 = 13.86 **	χ^2 = 1.35NS	χ^2 = 11.52**	χ^2 = 9.74 **	4.39 *	---	
* = (P<0.05) , ** = (P<0.01) , NS=Non-Significant differences						

The maternal exposure to AgNPs during pregnancy period in both doses effect on the number of embryos. The results showed highly significant decrease (P<0.01) in the numbers of the pre-implanted and post-implanted embryos in both doses compared to control group. Furthermore the higher dose shows more effect than the lower dose. (Table 6).

Table 6. Effect of AgNPs on number of embryos: pre and post implantation groups

Pre implantation			Post implantation	
No. of all embryos			No. of all embryos	
G1	G2	G3	G2	G3
Control	2mgAgNPs	20mgAgNPs	2mg	20mg
42	80	33	32	31
Chi-Square: χ^2 = 11.377 **				
** (P<0.01)				

The large size of AgNPs is less reactive in comparison to small size, small sizes; i.e. nanoparticles scale are predominantly considered under ROS generator group (21). The results showed that nanoparticles effects on the weights of mothers, could be attributed to these nanoparticles effects on appetite and nutrients absorption that effect on the supplemtation of necessary nutrients to fetuses. Previous researches on animals demonstrated that AgNPs causes severe accumulation and damage of GI system and hampers absorption with flow of nutrition to vital sight (24). The different size of AgNPs extended entrance into the human body mostly through oral portal route and separated in to other organ body and effects on weights of organism. Other study reversed bigger size AgNPs in colloidal form ingested directly through food material as medical devices and drugs causing disturbances of GI tract and malnutrition syndrome (18). On the other hand, this study reverse decreased in the weights of embryos after orally administrated of AgNPs to pregnant females during the gestation period. The umbilical cord is mostly made up of connective tissue well-known as (Wharton's Jelly) and has relatively few cells. This cord has one large umbilical vein and two umbilical arteries. These vessels transport blood to and from the placenta, where exchange between the mother and fetus takes place (21). In the present study, we observed the maternal and fetal nutrition rate after orally administration (for 21days) of AgNPs (2 and 20 mg/Kg/ B.Wt) was limited compare of control group. The influence toxicity of silver nanoparticles can be transported to the fetus by placental barrier. So, many animal revisions have shown that AgNPs can be enter the body and be transferred to the offspring (14). Furthermore, other groups demonstrated that AgNPs accumulate in the fetus through the placenta (16) and can be accumulate in the visceral yolk sac of pregnant mice (6). Another study, they also reported that the AgNPs concentration in the fetal brain was about 0.0035% of the dose ingested. Those conclusions that these nanoparticles effects on fetus and embryos brain and can be effects on their weights in pre implantation more than the post implantation when the blood brain barrier was not yet complete. This toxicity indicate that the potential exposure risk for AgNPs in pregnant women cannot be ignored (28). Other research showed that after exposure to AgNPs in pregnant mice, the offspring showed significant neural behavior changes such as spatial cognition disorders (27). All these evidence suggests that AgNPs may have severe toxic effects during neural differentiation and cause genotoxicity in the developmental process and previous developmental neurotoxicity in the earliest stages of embryonic development. The crown-rump length (CRL) is the measured length of the embryo in the midsagittal plane from the head to rump (3). The studies expected CRL has been reported as a risk factor for adverse perinatal products such as miscarriage (17), preterm birth and low birth weight (8). Therefore, the results showed decreased in CRL in different embryonic age indicate that these nanoparticles have toxicity on length and development of embryos. On the other hand, the higher dose of AgNPs is more effects on lengths of embryos, because the reproductive system of mammals, such as the ovaries and uterus, reveal periodic growth and reduction, which is strictly regulated by hormones. Therefore, dynamic activity and sternly hormonal control make this system more sensitive to foreign bodies and physiological stress compared to other physiological organs, so the misfortune of female reproduction certainly leads to abnormal fetal development. Many environmental chemicals have already demonstrated detrimental effects on the female reproductive system and already on embryonic development (5, 23).In the present study, the number of mothers (52 mothers) that have capable to produce embryos also effected by administration of Ag nanoparticles in low and high dose. The high dose of AgNPs is more achieved on production of embryos in mothers received 20mg/kg/ body weight, this could be attributed to the transfer of these nanoparticles through the placental barrier to embryos than the low dose that may be adverse on implantation of embryos; AgNPs in a low dose have not a complex effect on implantation of embryos compare of higher dose. On the other hand, some researchers showed that the numbers of nonviable fetuses

was significantly increased in dams treated to a single oral dose of 10 mg/kg/B.Wt AgNPs compared with the higher doses. This could be due to facilitation of aggregation AgNPs at higher concentration in gut and preventing internalization via the gastrointestinal tract and so reducing fetotoxicity at higher doses (19). The number of embryos that produce during pregnancy period also affected by dose of administration, the higher dose was more harmful effects on mothers and embryos. AgNPs may effect on implantation and already on number of embryos that embedded in uterus. Silver nanoparticles can be transport through the placental barrier during the embryogenesis after implantation and make adverse effect on number of implanted embryos and may be make reabsorption of embryos and reduced number of embryos in higher dose (46 embryos) compare of low dose (112 embryos). Other research investigates whether orally induce colloidal AgNPs reach after crossing mother mouse blood placental barrier and induce adverse effects in fetuses from treated group, and the research indicates bigger size nanosilver are able to reach to fetus after crossing the pregnant mouse blood placental barrier and portovenous circulation causing hamper of mother and fetus nutrition by obstruction of micro and macro channels (21). Other study displayed that acute intravenous exposure to AgNPs during late stages of pregnancy will increase vascular tissue contractility, potentially contributing to alterations in fetal growth (26). So, these nanoparticles may cause uterus contraction and lead to reabsorptions of embryos in pre or after implantation occur. Orally administration of silver nanoparticles AgNPs by using two concentration (2, 20 mg/kg/B.wt) can be transported to many vital organs such as brain during the embryological development and can be effects on mothers and embryos when given during the pregnancy period

REFRENCES

1. Abdel-Wahhab, M.; O. Joubert,; A. Y. Khadrawy; Safar, R.; A. El-Nekeety; C. Ronzani; H.G. Sawie, ; N.S. Hassan; L. Ferrari. and B.H. Rihn. 2017. Preliminary safety assessment of Eudragit®polymers nanoparticles administration in the rat brain. J. of Applied Pharmaceut. Sci.7 (07): 176-185

2. Abbott, N. J.; A. A. K. Patabendige; D. E. M. Dolman; S.R. Yusof and D.J. Begley. 2010. Structure and function of the blood–brain barrier. Neurobiol. Dis. 37(1): 13–25

3. ACOG, 2014. Society for maternal fetal medicine, american institute of ultrasound in medicine. Method for estimating due date. Obstet Gynecol , 611:1–4

4. Ali, N. S. and H. W.A. AL- Juthery. 2017. The application of nanotechnology for micronutrient in agricultureal production (review article). The Iraqi Journal of Agricultural Sciences,48(4): 984-990.

5. Armenti, A. E.; A.M. Zama; L. Passantino and M. Uzumcu. 2008. Developmental methoxychlor exposure affects multiple reproductive parameters and ovarian folliculogenesis and gene expression in adult rats. Toxicol. Appl. Pharm. 233: 286–296

6. Austin, C. A.; G.K. Hinkley ; A.R. Mishra; Q. Zhang; T.H. Umbreit; M.W. Betz; E.B. Wildt.; B.J. Casey; S. Francke-Carroll; S.M. Hussain; S.M. Roberts; K. M. Brown and P.L. Goering.2016.Nanotoxicology,10:654

7. Betzer, O., M. Shilo, R. Opochinsky, E. Barnoy, M. Motiei, E. Okun, G. Yadid and R. Popovtzer. 2017. The effect of nanoparticle size on the ability to cross the blood–brain barrier: an in vivo study. Nanomedicine, 12(13): 1533-1546

8. Bukowski, R.; G.C.S. Smith; F.D. Malone; R.H. Ball; D.A. Nyberg ; C.H. Comstock and S.D. Craigo. 2007. Fetal growth in early pregnancy and risk of delivering low birth weight infant: prospective cohort study. Br Med J.334(7598):836

9. Charehsaz , M.; K.S. Hougaard; H. Sipahi; A. I. D. Ekici; Ç. Kaspar; M. Culha; Ü. Ü. Bucurgat and A. Aydin. 2016. Effects of developmental exposure to silver in ionic and nanoparticle form: A study in rats. DARU. Journal of Pharma. Sciences. 24:24

10. Dănilă, O. O.; A.S. Berghian; V. Dionisie; D. Gheban; D. Olteanu; F. Tabaran; I. Baldea ; G. Katona ; B. Moldovan; S. Clichici; L. David and G.H. Filip. 2017. The effects of silver nanoparticles on behavior, apoptosis and nitro-oxidative stress in offspring Wistar rats. Nanomedicine. 12(12): 1455-1473

11. Hong, J.S.; S. Kim.; S.H. Lee; E. Jo, ; B. Lee; J. Yoon and M.Y. Lee. 2014. Combined repeated-dose toxicity study of silver

nanoparticles with the reproduction/ developmental toxicity screening test. Nanotoxicology 8:349-362
12. Hu, Y. L. and J.Q. Gao. 2010. Potential neurotoxicity of nanoparticles. Int. J. Pharm. 394: 115-121
13. Hussein, N.N. and A. H. Muslim. 2019. Detection of the antibacterial activity of AgNPs biosynthesized by *Pseudomonas aeruginosa*. The Iraqi Journal of Agricultural Sciences. 50 (2): 617- 625
14. Lee, Y.; J. Choi; P. Kim; K. Choi; S. Kim; W. Shon and K. Park . 2012. A transfer of silver nanoparticles from pregnant rat to offspring. Toxicol. Res. 28:139–141
15. Liu P; Z. Huang and N. Gu. 2013. Exposure to silver nanoparticles does not affect cognitive outcome or hippocampal neurogenesis in adult mice. Ecotoxicol. Environ. Saf. 87: 124–130
16. Melnik, E. A.; Y.P. Buzulukov; Demin, V. F.; Demin, V. A.; Gmoshinski, I. V.; Tyshko, N. V. and Tutelyan, V. A. 2013. Transfer of silver nanoparticles through the placenta and breast milk during in vivo experiments on rats. Acta Naturae, 5(3): 107–115
17. Mukri, F.; T. Bourne; C. Bottomley, ; C. Schoeb; E. Kirk and A.T. Papageorghiou. 2008. Evidence of early first trimester growth restriction in pregnancies that subsequently end in miscarriage. Br. J. Obstet Gynaecol,115:1273-1278
18. Oberdorster, G. and A. Maynard. 2005. ILSI Research Foundation/Risk Science Institute Nanomaterial Toxicity Screening Working Group. Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. Part Fibre. Toxicol. 2: 8
19. Philbrook, N. A.; L.M. Winn; A.N. Afrooz; N.B. Saleh and V.K. Walker. 2011. The effect of TiO2 and Ag nanoparticles on reproduction and development of *Drosophila melanogaster* and CD-1 Mice. Toxicol. Appl. Pharmacol.257:429–436
20. Project on Emerging Technologies. 2014. Consumer products inventory [Online]. Washington, DC: Project on Emerging Technologies. Available at: http://www.nanotechproject.org/cpi
21. Prakash, P. J and S. Royana. 2017. Bigger size silver nanoparticles hamper mother and fetus nutrition after serial ingestion of bolotic colloidal nano silver stirring solution through oral route: A revolutionary research discovery in teratogenicity and toxicity field. J. Nutr. Disorders Ther. 7: 221
22. SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA
23. Sun, J.; Q. Zhang; Z. Wang and B. Yan. 2013. Effects of nanotoxicity on female reproductivity and fetal development in animal models. International Journal of Molecular Sciences, 14(5): 9319-9337
24. Sung, J.; J. Ji; J.U. Yoon; D.S. Kim; M.Y. Song; J. Jeong and T.S. Kim. 2008. Lung function changes in Sprague-Dawley rats after prolonged inhalation exposure to silver nanoparticles. Inhal Toxicol. 20: 567-574
25. Sun, J.; Q. Zhang; Z. Wang and B. Yan. 2013. Effects of nanotoxicity on female reproductivity and fetal development in animal models. Int. J. Mol. Sci. 14: 9319-9337
26. Vidanapathirana, A. K.; L.C. Thompson; M. Herco; J. Odom; S.J. Sumner; T.R. Fennell and C.J. Wingard. 2018. Acute intravenous exposure to silver nanoparticles during pregnancy induces particle size and vehicle dependent changes in vascular tissue contractility in Sprague Dawley rats. Reproductive Toxicology, 75: 10-22
27. Wu, J.; C. Yu; Y. Tan; Z. Hou; M. Li; F. Shao and X. Lu. 2015. Effects of prenatal exposure to silver nanoparticles on spatial cognition and hippocampal neurodevelopment in rats. Environ. Res. 138:67–73
28. Yin, N.; B. Hu; R. Yang.; S. Liang, ; S. Liang and F. Faiola . 2018. Assessment of the developmental neurotoxicity of silver nanoparticles and silver ions with mouse embryonic stem cells in vitro. J. of Interdisciplinary Nanomedicine, 3(3): 133-145.