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EFFECTS OF SILVER NANOPARTICLES IN SOLUTION AND LIPOSOMAL FORM ON SOME BLOOD PARAMETERS IN FEMALE RABBITS DURING FERTILIZATION AND EARLY EMBRYONIC DEVELOPMENT

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ABSTRACT

Silver nanoparticles are the most rapidly growing classes of nanoproducts. In this study, we investigated the influence of subcutaneous injections of silver nanoparticles in solution and in liposomal form on hematological and biochemical parameters of blood of New Zealand White rabbits during hormonal treatment, fertilization and early embryonic development. The females treated by free silver nanoparticles and silver nanoparticles in liposomal form received silver at a dose of 10 µg/kg/day in 5 % glucose solution during 28 days. Blood sampling was done four times: the day before the compounds administration; on day 7 after the compounds administration; in the period after hormonal induction and fertilization and on the 14th day of pregnancy.
Our results showed changes in some biochemical (lactate dehydrogenase activities, progesterone and estradiol concentration, malondialdehyde level, *etc*.) and hematological (hematocrit, mean cell volume, mean corpuscular hemoglobin concentration, *etc*.) parameters under the influence of hormonal treatment and pregnancy. The concentration of progesterone showed significantly higher values ($P<0.05$) on GDs 1 in S group than in C group. The percentage of neutrophils was significantly higher in SG rabbits after 7 days of silver nanoparticles administration than that in the CG. There were no significant changes in red blood cells parameters, platelets, and activity of some ferments (ALP, AST, ALT, LDH, GGT) between control and silver groups during the entire period of experiment.
In conclusion, the hematological and biochemical values of blood obtained in the given study showed that free silver nanoparticles and silver nanoparticles in liposomal form in the investigated concentrations had no toxic effect on hormonal treatment, fertilization and early embryonic development in New Zealand White rabbits.

Keywords: Silver nanoparticles, rabbits, blood parameters

INTRODUCTION

Silver nanoparticles (AgNPs) are the most rapidly growing classes of nanoproducts. **(Song *et al.*, 2006; Luoma, 2008)**. It has been used for many years in different fields of medicine, biotechnology, and environmental technology as broad-spectrum antimicrobial agents **(Kim *et al.*, 2007; Kim *et al.*, 2008)**, in wound healing **(Lansdown, 2008; Tian *et al.*, 2007)**, in cancer therapy **(Safaepour *et al.*, 2009; Moaddab *et al.*, 2011)**, in biosensors **(Aslan, 2010)** or in biomaterials **(Samuel *et al.*, 2004; Rivero *et al.*, 2011)**. However, the detailed research of its effect on the environment, human and animal health has just begun. Several studies have shown toxic effect of AgNPs against mammalian cells through oxidative stress, DNA damage and chromosomal aberrations **(Asharani *et al.*, 2009; Carlson *et al.*, 2008; Park *et al.*, 2010; Braydich-Stolle *et al.*, 2010)**. The results of toxicity studies involving silver nanoparticles on zebrafish embryonic models show dose-dependent toxicity in embryos, which hinders normal development **(Kannan *et al.*, 2011; Asharani *et al.*, 2008)**. In contrast to cytotoxicity or embryonic studies using fish, in vivo studies using an animal model seemed to show relatively low toxicity **(Parka *et al.*, 2010; Park *et al.*, 2011)**. It has been reported that oral, inhalation or subcutaneously administered AgNPs are accumulated in some organs causing hepatotoxicity or renal toxicity **(Sung *et al.*, 2009; Kim *et al.*, 2010)**. Histopathological examinations indicated a dose-dependent increase of lesions related to silver nanoparticle exposure, including mixed inflammatory cell infiltrate, chronic alveolar inflammation and small granulomatous lesions **(Sung *et al.*, 2009)**. However, there were no significant toxicological changes during 28 days of AgNP inhalation in rats **(Hyun *et al.*, 2008)**. On the other hand, studies related to the influence of silver nanoparticles on wound healing show its role in metalloproteinase regulation, modulation of fibrogenic cytokines, reducing inflammation and favoring cellular apoptos is and cicatrization **(Tian *et al.*, 2007; Warriner *et al.*, 2005)**. AgNP effects are distinct from those of Ag^+ alone and depend on size and coating, indicating that AgNP effects are not due simply to the release of Ag^+ into the surrounding environment **(Powers *et al.*, 2011)**. There are only a few detailed studies concerning the influence of silver nanoparticles on animal reproductive performance **(Studnicka *et al.*, 2009; Park *et al.*, 2010)**. Little is known about the influence of silver nanoparticles on fertilization and early embryonic development in higher vertebrates. Furthermore, the mechanism of assimilation of AgNPs by the organism and its effect on various biochemical processes have not been clarified definitively.
The main purpose of this study was to investigate the influence of subcutaneous injections of silver nanoparticles in solution and in liposomal form (as targets of macrophages **(Kelly *et al.*, 2011)** on hematological and biochemical blood parameters of New Zealand White rabbits during hormonal treatment, fertilization and early embryonic development.

MATERIAL AND METHODS

Silver Nanoparticles Included in Liposomes

In our study we used silver nanoparticles with a size of 11 nm obtained by chemical reduction in ultra pure water **(Solomon *et al.*, 2007)**. For isotonic stabilization, we added glucose to obtain a final concentration of 50g/L (5 % solution). Lipids were added to the solution with nanoparticles in the proportion of 1 : 5. The suspension was sonicated for 5 minutes at room temperature. Liposomes were separated by centrifugation at 10000 g for 15 min. The size of liposomes was evaluated under microscope.

Animals and Experimental Design

Twelve female rabbits of New Zealand White strain were used in this study. The animals, weighing 4.3±0.3 kg (8 months old), were housed in individual cages, under controlled light/dark cycles (12L : 12D), and fed *ad libitum* with a commercial pelleted diet. The rabbits were divided into 3 groups. The does of the control group (CG) were subjected to subcutaneous injections of 5 % glucose solution during 28 days. Females of silver (SG) and liposome silver group (LSG) were subjected to subcutaneous injections of free silver nanoparticles and silver nanoparticles in liposomes, respectively, at a dose of 10 µg/kg/day in 5 % glucose solution during 28 days.

After 18 days of the compounds administration, the artificial insemination was performed in all groups of animals after the appropriate hormonally treatment. We used 40 IU of PMSG (Follimag, Intervet, Holland) for the synchronization of the cycle (injected 48 h before AI) and 20 µg/doe of GnRH (Gonadotropin-releasing hormon) (Fertagil, Intervet, Holland) for the ovulation induction (injected at the moment of insemination). Rabbits were fertilized intravaginally with 10×10^6 spermatozoa/doe in 0.5 ml of tris-citrate diluent. All rabbits were observed daily for their general condition, while the occurrence of abortions and number of fetuses were assessed on GD 14 and after birth (Fig. 1).

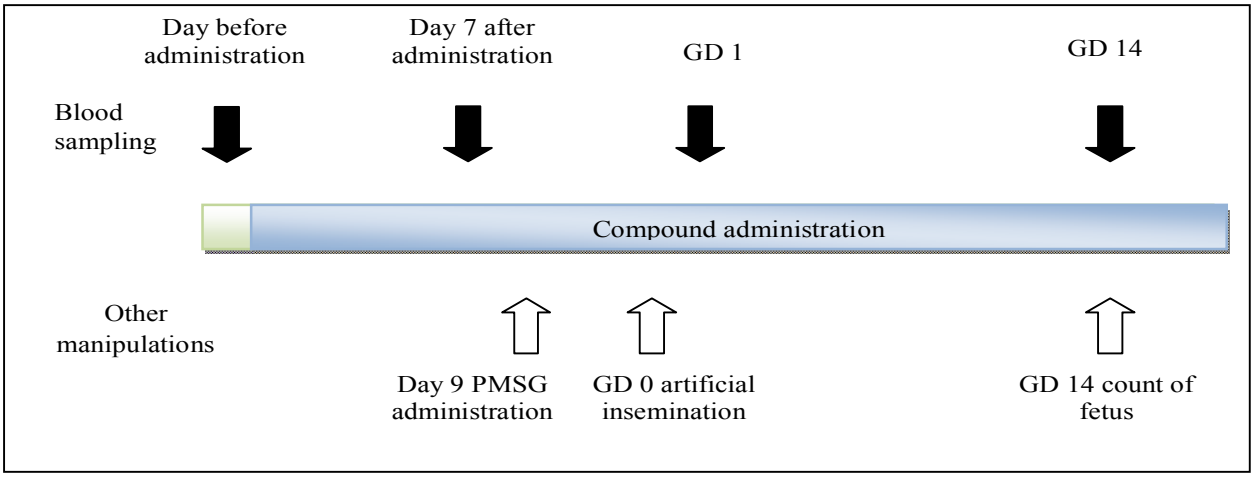

Figure 1 Experimental design

Haematological and blood chemical examinations

Blood sampling was done four times (Fig. 1): 1 – the day before the compound administration (Days 0); 2 – on day 7 after compound administrations (Days 14); 3 – in the period after hormonal induction and fertilization (GDs 1); and 4 – on day 14 of pregnancy (GDs 14). Blood samples were collected from the marginal ear vein of each animal into labeled vacutainers (Venosafe, Terumo Europe n.v). Initially, 2 ml of blood was collected into vacutainers with ethyldiaminetetracetic acid (EDTA) for measurement or calculation of hematological parameters (red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), platelets (PTL), mean platelet volume (MPV), platelet crit (PCT) and platelet distribution width (PDV), according to the method using diagon D-cell 60 auto hematologic analyzer. Differential WBC was determined by manual examination of blood smears. Other blood samples (4.5 ml) were collected into tubes without coagulant with gel for serum separation, kept at room temperature for about 30 min and centrifuged at approximately 1500 g for 15 min, and the serum samples were used to determine the following parameters: oestradiol (E_2), progesterone (PROG), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), glutamyl transferase (GGT), total protein (Tpr), albumin (Alb) and blood urea-nitrogen (BUN) using biochemical and enzyme immunoassay analyzer and commercially available Kit according to manufacturer's instruction (DRG Instruments GmbH; Germany, Human GmbH, Germany). The ratio of globulins fractions (α1, α2, β and γ) was measured using electrophoresis on cellulose acetate plates. Approximately 2 ml of blood was collected in a heparinized tube centrifuged at approximately 1500 g for 15min, and plasma obtained was examined for the malondialdehyde (MDA) levels by thiobarbituric acid method, as described previously **(Ohkava *et al.*, 1979)**. All statistical analyses were performed using the Minitab15 English statistical software package. The mean value and SEM were calculated for each of the measured parameters. Differences between groups were determined by nonpaired, 2-tailed Student t tests.

RESULTS AND DISCUSSION

It was shown that the diameter of obtained liposomes is about 200 nm (Fig. 2). This diameter is fully acceptable for *in vivo* conditions as a target of macrophages **(Kelly *et al.*, 2011)**.

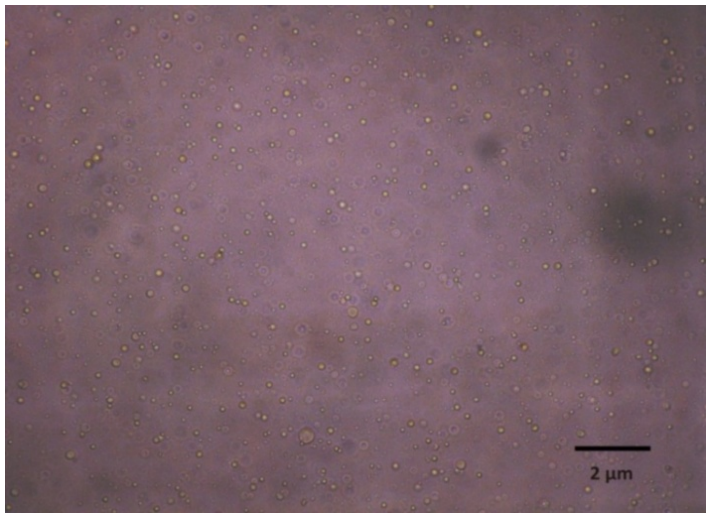

Figure 2 Liposomes with AgNPs

In this study, the effects of administration of AgNPs and AgNPs in liposomal form (10 µg/kg/day from Day 0 to GD 14) on blood parameters during hormonal treatment, fertilization and early embryonic development in New Zealand White rabbits were investigated. The parameters of red blood cells are shown in Table 1. Ht and MCV significantly increased, while MCHC significantly decreased in all investigated groups after hormonal stimulation (GD 1). These changes might reflect an increased LDH activity after gonadotropins surge. However, there were no significant changes in all parameters between control (CG) and silver groups (SG, LSG).

Table 1 Changes in red blood cell parameters in each group of rabbits

Day	RBC (x10^{12}/L)	Hb (g/L)	Ht (%)	MCV (fL)	MCH (pg)	MCHC (g/l)	RDW-CV (%)
CG							
Day 0	5.32±0.37	113.75±6.34	36.58±2.12	69.08±1.30	21.43±0.36	310.5±1.19	15.50±0.34
Day 7	5.21±0.24	113.33±2.80	36.53±0.74	70.37±2.23	21.73±0.64	309.7±0.90	15.73±0.29
GD 1	5.41±0.23	115.0±2.68	44.65±2.90	82.88±2.23↑↑	21.25±0.47	257.0±1.22 ↓↓↓	15.43±0.23
GD 14	5.23±0.69	112.70±11.3	35.00±4.65	67.07±1.25	21.73±0.85	324.7±12.7	14.50±0.78
SG							
Day 0	5.32±0.12	112.75±1.25	36.28±0.74	68.33±0.89	21.18±0.27	310.5±3.07	15.55±0.47
Day 7	5.18±0.18	111.0±3.85	35.50±1.50	68.55±0.94	21.38±0.30	312.8±3.50	15.6±0.72
GD 1	5.13±0.17	109.0±4.10	43.05±2.00 ↑	83.95±1.57 ↑↑↑	21.18±0.13	253.3±5.30 ↓↓↓	16.15±0.82
GD 14	5.70±0.26	121.0±5.03	38.73±1.53	68.00±0.65	21.17±0.19	311.7±0.88	14.53±0.27
LSG							
Day 0	5.22±0.12	115.5±2.72	37.20±0.39	71.43±1.05	22.10±0.50	309.8±4.98	15.48±0.1
Day 7	5.04±0.14	112.0±1.78	36.00±0.60	71.63±1.33	22.20±0.50	310.5±2.50	15.73±0.38
GD 1	5.17±0.19	114.5±2.50	46.18±1.70 ↑↑	89.60±2.17 ↑↑↑	22.15±0.41	247.3±6.5 ↓↓↓	15.43±0.53
GD 14	5.41±0.16	119.0±2.69	38.30±0.80	71.03±1.37	22.03±0.28	311.0±2.12	14.53±0.09

Legend: ↑: significantly high value, P<0.05, ↑↑: significantly high value, P<0.01 ↑↑↑: significantly high value, P<0.001, ↓↓↓: significantly low value, P<0.001 (different from the day before the compound administration) (Student's t-test).

Sequential changes of WBC parameters are shown in Table 2. The percentage of neutrophils was significantly higher in SG rabbits after 7 days of silver nanoparticles administration than those in the CG. The increase in the number of neutrophils occurred due to the reduction of eosinophils percentage. Silver nanoparticles might affect the immune system through the modulation of cytokine expression (increased IL-10, IL-1, IL-4, IL-10, IL-12, VEGF, IFN-γ, Ig E, increased or decreased IL-6 and TGF-β) **(Parka *et al.*, 2010; Tian *et al.*, 2007)**.

However, exposures to concentrations lower than 3 ppm did not produce any significant decrease of cytokine production **(Greulich *et al.*, 2009; Shin *et al.*, 2007)**. During the experimental period, the percentage of eosinophils decreased in all groupsof animals (significant changes were only in CG and in SG on Day 7 and GD1). On day 14 of pregnancy, the number of neutrophils increased (significant changes were only in CG and SG) at the expense of a decreased percentage of lymphocytes (but with no significant difference). The changes might reflect the inhibition of the immune system and the severity of stress during early pregnancy **(De Rijk *et al.*, 2002)**.

Table 2 Changes in white blood cell (WBC) parameters in each group of rabbits

Day	WBC (x10^{9}/L)	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophiles (%)
CG						
Day 0	10.45±0.17	37.00±0.91	43.00±2.68	5.25±0.63	11.75±1.55	3.00±0.91
Day 7	10.18±0.37	34.00±0.71 ↓	51.00±1.35 ↑	3.25±0.25 ↓	8.00±1.00	3.50±0.50
GD 1	11.13±0.52	36.00±1.58	47.50±1.50	3.00±0.71 ↓	9.75±0.95	3.75±0.48
GD 14	10.50±0.25	46.00±1.87 ↑	35.00±3.18	2.67±0.88 ↓	13.25±1.65	3.00±0.40
SG						
Day 0	9.63±0.69	34.75±2.56	42.00±3.14	4.75±0.48	12.00±2.38	6.50±0.65
Day 7	8.28±0.93	40.00±2.00 *	47.50±2.18	2.25±0.25↓↓↓*	7.25±1.00	3.00±0.40 ↓↓
GD 1	10.33±0.78	35.50±1.66	48.50±3.66	2.00±0.41 ↓↓	9.25±1.11	4.25±1.10
GD 14	9.77±1.30	49.75±4.92 ↑	32.5±5.33	4.25±0.85	9.75±0.95	3.75±0.25 ↓↓
LSG						
Day 0	9.13±0.48	43.50±1.50	38.75±2.25	4.75±0.85	10.29±1.49	2.75±0.64
Day 7	10.40±1.03	37.25±4.00	49.75±4.92	4.00±0.58	8.25±1.38	3.00±0.41
GD 1	13.10±2.13	40.00±3.75	43.25±5.02	3.50±0.96	8.04±0.96	4.25±0.85
GD 14	11.00±0.42 ↑	44.75±4.94	34.25±2.87	4.25±0.63	11.25±1.31	3.00±2.08

Legend: ↑: significantly high value, P<0.05, ↓: significantly low value, P<0.05 ↓↓: significantly low value, P<0.01, ↓↓↓: significantly low value, P<0.001 (different from the day before the compound administration) *: significantly different from the control group, P<0.05 (Student's t-test).

The treatment by silver nanoparticles did not influence platelet parameters during the entire experimental period (Tab 3). But on day 14 of gestation, the number of platelets decreased (p<0.05) and PDV significantly increased in the control group (p<0.05). This change might reflect an increase of peroxidase products (MDA tended to increase in all groups on day 14 of pregnancy, and in the LSG there was a significant difference as compared with Day 0) causing platelets clumping **(Walsh, 1994)**. However, in SG and LSG, the PDV value showed almost no significant changes. In vivo studies using mouse models showed antiplatelet properties of nanosilver. **Shrivastava *et al.* (2009)** found that nano-Ag inhibited platelet aggregation in a dose-dependent manner in whole blood through changes in signaling of aggregation. The platelets level was increased in CG (p<0.05) and SG (no significant difference) on day 14 of pregnancy, as compared with the day before the compound administrations.

Table 3 Changes in platelets parameters and peroxidase products in each group of rabbits

Day	PTL (x10^{9}/L)	MPV (fl)	PDV	PCT (%)	Gpx (AU/mL)	MDA (mmol/L)
CG						
Day 0	388.8±30.0	5.95±0.28	14.70±0.20	0.231±0.024	15.28±1.30	0.250±0.052
Day 7	396.3±52.9	5.93±0.59	14.90±0.40	0.240±0.054	9.38±1.62 ↓	0.237±0.050
GD 1	425.0±30.1	7.13±0.56	15.35±0.28	0.300±0.024	12.13±0.71	0.167±0.039
GD 14	258.7±29.2 ↓	7.33±0.52	16.03±0.32 ↑	0.186±0.012	10.53±1.11 ↓	0.385±0.042
SG						
Day 0	332.5±44.3	6.83±0.54	15.45±0.39	0.220±0.017	12.98±0.90	0.321±0.027
Day 7	395.5±26.4	6.30±0.29	15.25±0.28	0.248±0.013	18.60±5.18	0.199±0.019 ↓
GD 1	402.3±84.5	7.40±0.31	15.70±0.33	0.296±0.065	15.07±1.20	0.154±0.046 ↓
GD 14	208.0±51.6	7.53±1.07	16.70±0.90	0.150±0.018 ↓	10.20±0.20 ↓	0.453±0.060
LSG						
Day 0	284.0±57.5	7.13±0.36	15.95±0.48	0.202±0.038	19.60±0.76	0.150±0.035
Day 7	284.0±103.1	7.28±0.39	16.20±0.40	0.195±0.061	12.50±4.30	0.244±0.022
GD 1	223.5±105.7	8.28±0.19 ↑	16.25±0.25	0.185±0.088	10.88±1.73 ↓↓	0.257±0.040
GD 14	286.8±58.4	7.45±0.60	16.25±0.64	0.207±0.036	10.25±1.20 ↓↓↓	0.397±0.064↑↑↑

Legend: ↑: significantly high value, P<0.05, ↑↑↑: significantly high value, P<0.001, ↓: significantly low value, P<0.05 ↓↓: significantly low value, P<0.01 ↓↓↓: significantly low value, P<0.001 (different from the day before the compound administration) (Student's t-test).

There were no significant differences in biochemical values of blood among the groups (Tab 4), and no significant differences in the ratio of globulins fractions (Tab 5).

Silver ions show a high affinity for the thiol group in the liver that can lead to hepatotoxicity **(Drake, 2005)**. Our results of biochemical parameters in the silver group are similar to those obtained by **Sung *et al.*(2009). Kim *et al.* (2010)** showed a significant increase (P <0.01) in alkaline phosphatase activity (ALP) and a significant decrease in protein concentration in female rats after 90-day oral administration of silver nanoparticles in the dose of 125 and 500 mg/kg.

According to previous publications using mice in vivo, it was reported that levels of AST, ALT and ALP were significantly increased after the oral administration of 1mg/kg of AgNPs for 28 days. However BUN, creatinine, Tpr and Alb were not increased, but inflammatory responses were observed in the kidney. These results mean that bare AgNPs may induce hepatotoxicity and kidney damage at a repeated oral administration **(Parka *et al.*, 2010).**

Table 4 Changes in biochemical parameters of blood in each group of rabbits

Day	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	GGT (IU/L)	Crea (µmol/L)	BUN (mmol/L)
CG							
Day 0	148.69±31.30	32.50±5.56	65.41±3.36	148.75±17.99	8.25±1.39	153.4±4.05	26.38±0.23
Day 7	150.45±36.65	25.75±1.80	74.54±5.00	134.70±16.07	6.11±1.30	163.77±8.86	26.78±0.26
GD 1	147.50±22.70	34.00±9.18	60.30±6.45	436.80±54.72 ↑↑	6.55±1.57	177.03±6.44↑	26.68±0.13
GD 14	100.35±21.78	21.00±3.60	64.38±2.09	137.25±12.57	7.38±1.52	124.25±3.74↓	28.03±1.14
SG							
Day 0	141.83±37.30	22.50±3.48	65.00±7.50	113.88±7.63	9.60±1.15	156.85±11.35	26.1±0.74
Day 7	146.85±24.00	29.75±5.22	81.60±9.58	118.50±20.10	8.88±1.23	165.66±8.65	25.65±0.21
GD 1	128.70±19.20	30.67±2.90	72.70±9.75	385.05±58.00 ↑↑	7.13±0.89	184.58±9.70	26.05±0.56
GD 14	90.13±20.20	20.25±3.77	66.99±3.99	136.10±9.20	7.10±1.37	129.80±6.66	27.23±0.58
LSG							
Day 0	154.33±44.85	37.00±3.49	65.30±12.7	116.90±8.59	9.47±1.60	143.35±3.09	27.12±1.26
Day 7	147.00±48.00	29.50±4.52	74.17±17.3	104.30±14.20	8.38±1.27	145.30±5.8	27.00±0.74
GD 1	147.50±48.00	36.00±1.29	64.38±10.9	421.60±100.50 ↑	10.38±0.61	164.70±5.62↑	26.80±0.86
GD 14	104.65±31.00	30.25±5.12	74.25±6.29	160.80±12.30 ↑	9.90±1.26	118.38±6.86↓	27.18±1.15

Legend: ↑: significantly high value, P<0.05, ↑↑: significantly high value, P<0.01, ↓: significantly low value, P<0.05 (different from the day before the compound administration) (Student's t-test)

LDH activities and Crea was significantly increased in all groups after the artificial insemination. These changes might reflect gonadotropins surge related to GnRH treatment **(Zimin *et al.*, 1992)**. Silver nanoparticles treatment did not influencethe changes in the ratio of globulins fractions during all experimental period (Tab 5).

Serum estradiol concentration increased in all groups of animals on day 14 of gestation (Tab 6). But there were no significant differences in its concentration between the control (CG) and silver groups (SG, LSG) during the experimental period.

Table 5 Changes in the ratio of globulins fractions in each of the rabbits group

Day	Tpr (g/L)	Alb (%)	α1 (%)	α2 (%)	β (%)	γ (%)
CG						
Day 0	57.43±0.66	46.10±0.99	7.35±0.25	6.93±0.39	17.20±0.37	22.43±1.01
Day 7	58.05±1.32	46.58±1.44	8.18±0.47	7.28±0.54	15.80±0.50	22.18±0.21
GD 1	57.40±0.96	46.60±1.14	7.85±0.91	6.05±0.98	18.40±1.73	21.10±1.32
GD 14	55.38±0.81	50.20±1.89	7.78±1.13	6.38±0.85	16.83±1.75	18.83±0.97↓
SG						
Day 0	57.65±1.99	45.53±1.90	6.55±0.36	6.58±0.22	16.58±0.61	24.78±2.51
Day 7	58.08±3.18	44.78±2.11	7.60±0.34	7.20±0.80	16.08±1.52	25.10±2.98
GD 1	58.63±3.00	44.83±1.22	7.30±0.47	6.18±0.50	18.28±1.14	22.68±0.85
GD 14	53.35±1.12	49.50±1.28	6.98±0.44	6.13±0.57	17.25±1.16	20.15±0.90
LSG						
Day 0	59.40±2.28	45.68±0.93	8.35±0.64	7.60±0.38	17.93±0.26	20.45±0.73
Day 7	58.85±1.20	45.73±0.43	7.63±0.59	7.28±0.27	16.90±0.54	22.55±1.03
GD 1	59.13±1.32	45.48±1.43	7.08±0.15	5.78±0.17↓↓	18.83±0.57	22.85±1.26
GD 14	55.73±1.10	49.03±2.11	7.43±0.76	6.85±0.70	16.70±1.41	20.00±0.56

Legend: ↓: significantly low value, P<0.05 ↓↓: significantly low value, P<0.01 (different from the day before the compound administration) (Student's t-test)

Table 6 Changes in estradiol and progesterone concentrations in each group of rabbits

	E_2 (pmol/L)				PROG (nmol/L)			
	Day 0	Day 7	GD 1	GD 14	Day 0	Day 7	GD 1	GD 14
CG	81.31±12.5	80.03±9.10	78.14±6.35	119.70±13.0 ↑	0.383±0.069	0.324±0.075	7.56±0.75 ↑↑↑	64.3±6.70 ↑↑↑
SG	71.32±7.18	74.00±6.80	80.06±7.25	124.15±4.50 ↑↑↑	0.199±0.067	0.258±0.056	12.49±2.36 ↑↑↑ *	75.88±3.70 ↑↑↑
LSG	67.95±4.00	68.37±2.15	69.33±4.30	129.10±11.0 ↑↑	0.373±0.061	0.350±0.055	6.72±0.92 ↑↑↑	72.47±1.70 ↑↑↑

Legend: ↑: significantly high value, P<0.05, ↑↑: significantly high value, P<0.01, ↑↑↑: significantly high value, P<0.001, (different from day before compound administration) *: significantly different from the control group, P<0.05 (Student's t-test)

The progesterone grew markedly in all groups after artificial insemination, and on day 14 of pregnancy it was considerably higher than on Day 0 and on GD 1. However, the concentration of progesterone showed significantly higher values (P<0.05) on GDs 1 in SG than CG (Tab 6). This can be accounted for by a greater number of fetuses per doe in SG than in CG (Tab 7).

Table 7 Fertility, kindling rate and ratios of total and live born by kindling females in each group of rabbits

Groups of animals	Number of inseminated females	Kindling females (%) (number)	Number of fetuses per doe (%) (number)	Number of born per doe (%) (number)
CG	6	5 (83.33)	5.75±1.3	5.75±1.3
SG	6	6 (100)	7.50±1.6	7.20±1.2
LSG	6	5 (83.33)	6.60+1.7	6.60+1.7

In the present study, the concentration of nanoparticles was low (10 µg/kg/day), but in the studies on Zebrafish embryos models, the AgNPs showed toxic effects at lesser concentrations **(Lee *et al*., 2007)**. Experiments carried out on zebrafish embryos **(Lee *et al.*, 2007)** have shown that individual silver nanoparticles can passively diffuse into developing embryos via chorionic pore canals, accumulate inside the body, create specific negative effect on embryonic development and selectively generate atypical phenotypes in a dose-dependent manner. According to the previous publications using rats, it was demonstrated that AgNPs reduces the number of primary follicles and caused inhibition of ovulation at doses 1ppm and 10 ppm via intraperitoneal injection **(Ghorbanzadeh *et al.*, 2011)**. In the present study no negative effect of nanoparticles on kindling rate, fetus survival, and number of born per doe was detected (Tab 6). Furthermore, in rabbits treated with AgNPs the number of fetuses per female tended to increase, but with no significant difference.

Wijnhoven *et al.* (2009) advanced a hypothesis that the toxic effects of silver are proportional to free silver ions number, **Kim *et al.* (2009)** however, showed that the effects of silver nanoparticles are not accounted for only by the ionization of silver from the surface of silver nanoparticles, but may derive (at least in part) from direct effects of nanoparticles. In fact, the biological action of AgNPs was associated with the presence of bare metallic nanoparticles surfaces and its ability to bind with blood proteins **(Kittler *et al*., 2009)**. In our study we used silver nanoparticles in solution and liposomal form. On the basis of our very limited data, we hypothesized that AgNPs in liposomal form has a different mechanism of action, the path of assimilation and a less toxic effect on biological processes, as compared with free AgNPs in solution.

CONCLUSION

AgNPs are promising for the treatment of infertility associated with infection. In fact, infections may be associated with up to 40% of spontaneous preterm births, especially those taking place at an early gestational age **(Gibbs *et al.*, 1992)**.

In conclusion, the hematological and biochemical parameters of blood obtained in the given study showed that free silver nanoparticles and silver nanoparticles in liposomal form in investigated concentration had no toxic effect on hormonal treatment, fertilization and early embryonic development in New Zealand White rabbits.

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