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SILVER NANOPARTICLES STIMULATE SPERMATOGENESIS IMPAIRMENTS IN TESTIS AND EPIDIDYMIS

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ABSTRACT

This research focuses on the further study of new effects of hybrid nanocomposites based on branched copolymers of dextran-polyacrylamide in nonionic, D-g-PAA and anionic D-g-PAA(PE) form, with included silver nanoparticles (AgNP) on the male reproductive function using male mammals (mice), which has not been studied before. D-g-PAA (0,172 mg/ml), D-g-PAA(PE) (0,172 mg/ml), D-g-PAA/AgNPs (0,092 mg-Ag/ml), D-g-PAA(PE)/AgNPs (0,092 mg-Ag/ml) saline solution were introduced by intratesticular injection (in 0,2 mL) once a day, five times. For the first time it was shown, that under conditions of five times treatment with D-g-PAA(PE)/AgNPs (silver nanoparticles in the polymeric matrix D-g-PAA(PE)) were found the disorder of reproductive function in male mice: significant changes in the viability and death of spermatozoa and of spermatocytes (primary), as well as an increase in pre- and post-implantation embryonic mortality rates. However, under conditions of treatment with D-g-PAA and D-g-PAA(PE), as well as D-g-PAA/AgNPs, no significant changes in the values of 1) number of live newborns (pups) per female; 2) pre- and post-implantation mortality rates of embryos were established; 3) sperm and abnormal sperm forms (%); 4) spermatocytes (primary) and spermatids (%); 5) living, apoptotic and necrotic cells in the testicular cells (spermatocytes (primary)) and in the epididymis cells (spermatozoa). The data obtained indicate that branched star-like polymers in anionic form with incorporated silver nanoparticles (D-g-PAA(PE)/AgNPs) stimulate spermatogenesis impairments in testis and epididymis of male mice. Our data suggest that treatment with such silver nanosystems (silver nanoparticles in the polymer matrix D-g-PAA(PE)) are not critically dangerous for therapeutic use and requires further study.

KEYWORDS: Polymer matrix, Silver nanoparticles, Male reproductive function, Testis, Epididymis, Sperm.

INTRODUCTION

Recently, a number of outstanding applications of the nanomedicine (chemotherapeutic agents, biological agents, immunotherapeutic agents etc.) in the treatment of various diseases has increased.[1,2]

It has been proved that polymers with a dextran core and grafted polyacrylamide chains dextran-polyacrylamide (D-*g*-PAA) are effective in photodynamic chemotherapy, which gives confidence in the prospect of drug-nanosystem.[3,4]

Previously, we obtained data on the effect of D-*g*-PAA polymers on male and female reproductive function in mice.[5-7]

This research focuses on the further study of new effects of hybrid nanocomposites based on branched copolymers of dextran-polyacrylamide in nonionic, D-*g*-PAA and anionic D-*g*-PAA(PE) form, with included silver nanoparticles (AgNP) on the male reproductive function using male mammals (mice), which has not been studied before.

The aim is to evaluate the effect of a five-time treatment of branched star-like polymers with a dextran core and grafted polyacrylamide chains in non-ionic (D-*g*-PAA) and anionic (D-*g*-PAA(PE)) form with incorporated silver nanoparticles (D-*g*-PAA)/AgNPs and D-*g*-PAA(PE)/AgNPs) on the male reproductive function, namely: 1) under condition of coupling such males with intact females to evaluate: number of live newborns

(pups) and pre- and post-implantation embryonic mortality rates; and 2) on the cells in testis and epididymis of such male mice: number of sperm (concentration of sperm (millions/ml)) and the number of abnormal sperm (%); ratio of cells of different generations of spermatogenic epithelium (%) in the testes; number of living, apoptotic and necrotic cells of the testis (spermatocytes (primary)) and the number of living, apoptotic and necrotic cells of the epididymis of the spermatozoa), which has not been studied before.

MATERIALS AND METHODS

***Polymer matrices*.** As a nanocarrier we used a branched copolymer obtained by grafting polyacrylamide (PAA) chains onto dextran (M_w=7×10^4, g×mol^{-1}) backbone using a ceric-ion-reduce initiation method.[8]

This redox process initiates free radical sites exclusively on the polysaccharide backbone, thus preventing from the formation of homopolymer polyacrylamide. The detail of synthesis, identifications and analysis of internal polymer structure were described in.[9]

AgNPs/Polymer nanosystem synthesis. AgNPs were synthesized by reduction of Ag precursor ($AgNO_3$) dissolved in polymer solution. 2 ml of a 0.1M $AgNO_3$ aqueous solution was added to 5 ml of aqueous polymer solution (C=1×10^{-3} g×cm^{-3}) and stirred during 20 min. Then, 2 ml of 0.1 M aqueous solution of $NaBH_4$ was added. The final aqueous solution was stirred during 30 min. It turned reddish brown, thus the formation of AgNPs was indicated. The TEM investigation of silver sols has shown that NPs synthesized in uncharged D-*g*-PAA polymer matrix had the size of 8—15 nm. The silver sol synthesized in charged polymer matrix differ from the sols synthesized in non-charged matrix and had the size of 2—5 nm. TEM images demonstrated the differences in the sols obtained in the solutions of star-like uncharged and charged polymers and it can be explained by the chemical nature of both polymer matrices. The interaction of silver ions with the anionic (charged) polymer matrix takes place with both carbamide (as in uncharged polymers) and carboxylated groups. Obviously, on the carbamide groups of charged matrices the same particles form as in the uncharged matrices, particles formed on the carboxylated groups are smaller. Thus, the study of silver sols synthesized in branched polymer matrices of various natures has shown that AgNPs are spherical irrespective of the matrix nature. However, in the uncharged polymer matrix the particles with a size of 10—15 nm predominantly formed. In the charged polymer matrices the particles with a size less than 4 nm also observed. Details of synthesis, identification and analysis of silver nanoparticles have been described in.[10]

***The treatment of substances was carried out in the following way*:** D-*g*-PAA (0,172 mg/ml), D-*g*-PAA(PE) (0,172 mg/ml), D-*g*-PAA/AgNPs (0,092 mg-Ag/ml), D-*g*-PAA(PE)/AgNPs (0,092 mg-Ag/ml) saline solution were introduced by intratesticular injection (in 0,2 mL) once a day, five times.

Animals. Experiments (two series) have been conducted on 100 (50 male and 50 females) Albino white laboratory mice (weighing 25-30 g) in compliance with all requirements for work with laboratory animals (International European Convention for the Protection of Vertebrate Animals, Strasbourg, 1986). After the experiments, anesthetized by Nembutal animals were exterminated by cutting the spinal cord. The objective status of the animals (appearance, overall motor activity, need for food and water, and body weight) were evaluated before and during the experiment.

In the first series of experiments, animals were divided in to the same groups: I – physiological solution – control (N=5); II – D-*g*-PAA (N=5); III – D-*g*-PAA(PE) (N=5); IV - D-*g*-PAA/AgNPs (N=5); V – D-*g*-PAA(PE)/AgNPs (N=5); N is the number of animals in the group. On the third day after the treatment, males were planted to the females in a ratio of 1:2 (male/females). Coupling and subsequent manipulation of embryos were performed according to the Mank method (1990). Sampling of experimental material (ovaries, tubes, and uterus) was performed under anesthetic anesthesia for 10/11 days after replanting. The experiment was completed on day 24 after replanting the male with birth in control and experimental animal live newborns (pups).

In the second series of experiments, animals (males) were divided into the five groups treated with: I – physiological solution – control (N=5); II – D-*g*-PAA (N=5); III – D-*g*-PAA(PE) (N=5); IV - D-*g*-PAA/AgNPs (N=5); V – D-*g*-PAA(PE)/AgNPs (N=5); N is the number of animals in the group. On the third day after the last (fifth) injection of substances under nembutal anesthesia, the experimental material (testes and epididymides) was collected. The animals were removed from the experiment by cutting the spinal cord under anesthetic anesthesia, following the rules of euthanasia.

Sperm viability; Estimation of the number of sperm (concentration of sperm (millions/ml)) and the number of abnormal forms of sperm (%); Evaluation of the ratio of cells of different generations of spermatogenic epithelium (%); Embryonic mortality in mice; Method of color fluorescent dyes; Statistical analysis were performed and calculated as described by us earlier.[5]

RESULTS

Number of live newborns (pups) under conditions of treatment with silver nanoparticles in the polymer matrices. Under conditions of treatment with D-*g*-PAA(PE) and D-*g*-PAA(PE), no significant changes were established in the number of live newborns (pups), compared to such values in the control, respectively, 6,75±0,95 pcs/s (p>0,05, n=4) and 5,75±0,50 pcs/s (p>0,05, n=4) and in the control 7,25±0,95 pcs/s.

Number of live newborns (pups) under conditions of treatment with D-*g*-PAA/AgNPs was 6,25±0,50 pcs/s (p>0,05, n=4) and did not differ significantly compared to this value in the control of 7,25±0,95 pcs/s. A decrease in the number of live newborns (pups) was observed under conditions of treatment with D-*g*-PAA(PE)/AgNPs, 4,25±0,50 pcs/s (p<0,05, n=4) compared to the value in the control 7,25±0,95pcs/c, compared to the corresponding values under conditions of treatment with D-*g*-PAA(PE) and compared with the corresponding values under conditions of treatment with D-*g*-PAA/AgNPs.

Thus, under conditions of treatment with D-*g*-PAA(PE)/AgNPs, there is a decrease in the number of live newborns (pups) per female.

Pre- and post-implantation embryonic mortality. Under the conditions of coupling males that received polymer matrices, both D-*g*-PAA and D-*g*-PAA(PE), with intact females no significant changes in the values of pre- and post-implantation mortality of embryos were established (p>0,05, n=4), compared to this values in the control.

Under conditions of treatment with D-*g*-PAA/AgNPs, no significant changes in the pre- and post-implantation mortality rates of embryos were established (p>0,05, n=4), compared to this values in the control.

Under conditions of treatment with D-*g*-PAA(PE)/AgNPs an increase in the values of pre- and post-implantation mortality of embryos was established (p<0,05, n=4), compared with the corresponding values in the control.

Under conditions of treatment with D-*g*-PAA(PE)/AgNPs the values of pre- and post-implantation mortality of embryos increased (p<0,05, n=4) compared to the corresponding values under conditions of treatment with D-*g*-PAA(PE) and compared with the corresponding values under conditions of treatment with D-*g*-PAA/AgNPs.

Thus, under conditions of treatment with D-*g*-PAA(PE)/AgNPs, the values of pre- and post-implantation embryonic mortality in mice increase (Table 1).

Table 1: Embryonic mortality under the conditions of treatment with silver nanoparticles in the polymer matrices.

	Pre-implantation mortality (%)	**Post-implantation mortality (%)**
Control:	8,76±0,74	5,38±0,37
D-*g*-PAA	10,13±2,23	8,16±2,43
D-*g*-PAA(PE)	10,62±2,81	8,82±2,72
D-*g*-PAA/AgNPs	10,53±2,38	7,76±2,37
D-*g*-PAA(PE)/AgNPs	33,23±1,24 *#$	15,76±2,14 *#$
Notes: * - p<0,05 - probability differences in the average group data with respect to these variables in the control group animals; # - p<0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA(PE); $ - p<0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA/AgNPs; M ± σ.		

Number of sperm (concentration of sperm (millions /ml)) and the number of abnormal forms of sperm (%). No significant changes in sperm count and abnormal sperm forms (%) was observed under conditions of treatment with D-*g*-PAA, D-*g*-PAA(PE) and D-*g*-PAA/AgNPs - (p>0,05, n=4) compared with the same value in the control.

No significant changes in sperm counts were observed under conditions of treatment with D-*g*-PAA(PE)/AgNPs (p>0,05, n=4) compared with such a value in the control.

An increase in the number of abnormal forms of sperm (%) was found under conditions of treatment with D-*g*-PAA(PE)/AgNPs (p<0,05, n=4) compared to this value in the control.

An increase in the number of abnormal spermatozoa (%) under conditions of treatment with D-*g*-PAA(PE)/AgNPs was found compared to that value under conditions of treatment with D-*g*-PAA(PE) (p<0,05, n=4) and compared to that value in the group under conditions of treatment with D-*g*-PAA/AgNPs (p<0,05, n=4).

Thus, under conditions of treatment with D-g-PAA(PE)/AgNPs, an increase in the number of abnormal sperm forms (%) was observed both the number of sperm with total anomalies and number of sperm with primary anomalies (Table 2).

Table 2: Functional state of sperm under conditions of treatment with silver nanoparticles in the polymer matrixes.

	Concentration of sperm, (1×10^6/ml)	Number of abnormal sperm, %	
		Total	Primary anomalies
Control	36,4±1,29	17,6±1,70	5,8±0,50
D-*g*-PAA	35,8±1,50	19,4±1,89	6,8±0,50
D-*g*-PAA(PE)	35,4±0,95	21,4±1,63	7,8±0,95
D-*g*-PAA/AgNPs	34,23±1,29	22,4±1,73	6,6±0,50
D-*g*-PAA(PE)/AgNPs	34,40±1,29	33,8±1,50 *#$	10,60±0,50 *#$
Notes: * - p <0,05 - probability differences in the average group data with respect to these variables in the control group animals; # - p <0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA(PE); $ - p <0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA/AgNPs; M ± σ.			

***The ratio of cells of different generations of spermatogenic epithelium (%) in the testes*.** Under conditions of treatment with D-*g*-PAA and D-*g*-PAA(PE), no significant changes in the number of spermatocytes and in the number of spermatides (%) (p>0,05, n=4) were established compared with such value in the control.

Under conditions of treatment with D-*g*-PAA/AgNPs, there were no significant changes in the number of spermatocytes and in the number of spermatides (%) (p>0,05, n=4) compared to this value in the control.

A decrease in the number of spermatocytes (%) was registered under conditions of treatment with D-*g*-PAA(PE)/AgNPs (p<0,05, n=4) compared to that in the control. And under conditions there was found a decrease in the number of spermatids (%) (p<0,05, n=4) compared to this value in the control.

A decrease in the number of spermatocytes and in the number of spermatides (%) under conditions of treatment with D-*g*-PAA(PE)/AuNPs were observed compared to such value in the group D-*g*-PAA(PE) and to values in the group D-*g*-PAA/AgNPs (p<0,05, n=4).

Thus, under conditions of treatment with D-*g*-PAA(PE)/AgNPs there was a decrease in the number of spermatocytes (primary) and spermatides (%) on smears from homogenate of the testes (Table 3).

Table 3: The number of testicular spermatogenic cells under conditions of treatment with silver nanoparticles in the polymer matrices.

	Spermatogonia	Spermatocytes	Spermatides	Sertoli
Control	10,2 ±1,25	16,2 ±1,29	53,8±2,58	4,2 ±0,50
D-*g*-PAA	10,4 ±1,29	15,6±0,95	51,8±0,95	4,2±0,50
D-*g*-PAA(PE)	9,4±0,95	15,4 ±1,29	51,4±1,50	4,6±0,57
D-*g*-PAA/AgNPs	9,1±0,81	15,2 ±0,95	52,8±1,41	4,0±0,50
D-*g*-PAA(PE)/AgNPs	9,0±0,50	8,4 ±0,95*#$	26,8±0,81*#$	3,6±0,50
Notes: * - p <0,05 - probability differences in the average group data with respect to these variables in the control group animals; # - p <0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA(PE); $ - p <0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA/AgNPs; M ± σ.				

***The number of living, apoptotic and necrotic cells of the testis (spermatocytes (primary))*.** No significant changes were found in the numbers of living, apoptotic and necrotic cells of the testis (spermatocytes (primary)) under the conditions of treatment with both D-*g*-PAA and D-*g*-PAA(PE) (p>0,05, n=4) compared to this value in the control.

No significant changes in the numbers of living, apoptotic and necrotic cells of the testis (spermatocytes (primary)) were found under the conditions of treatment with D-*g*-PAA/AgNPs (p>0,05, n=4) compared to this value in the control.

Under the conditions of treatment with D-*g*-PAA(PE)/AgNPs there were found reduced number of living cells of the testis (spermatocytes (primary)) (p<0,05, n=4) and the number of apoptotic and necrotic cells of the testis increased (p<0,05, n=4) compared to the control.

Under conditions of treatment with D-*g*-PAA(PE)/AgNPs the number of living cells of the testicles (spermatocytes (primary)) reduced and the number of apoptotic increased (p<0,05, n=4) compared to the corresponding values under conditions of treatment with D-*g*-PAA(PE) and compared with the corresponding values under conditions of treatment with D-*g*-PAA/AgNPs.

Thus, under conditions of treatment with D-*g*-PAA(PE)/AgNPs there was a decrease in the number of living cells of the testicles (spermatocytes (primary)) and an increase of apoptotic cells on smears from homogenate of the testes (Fig. 1).

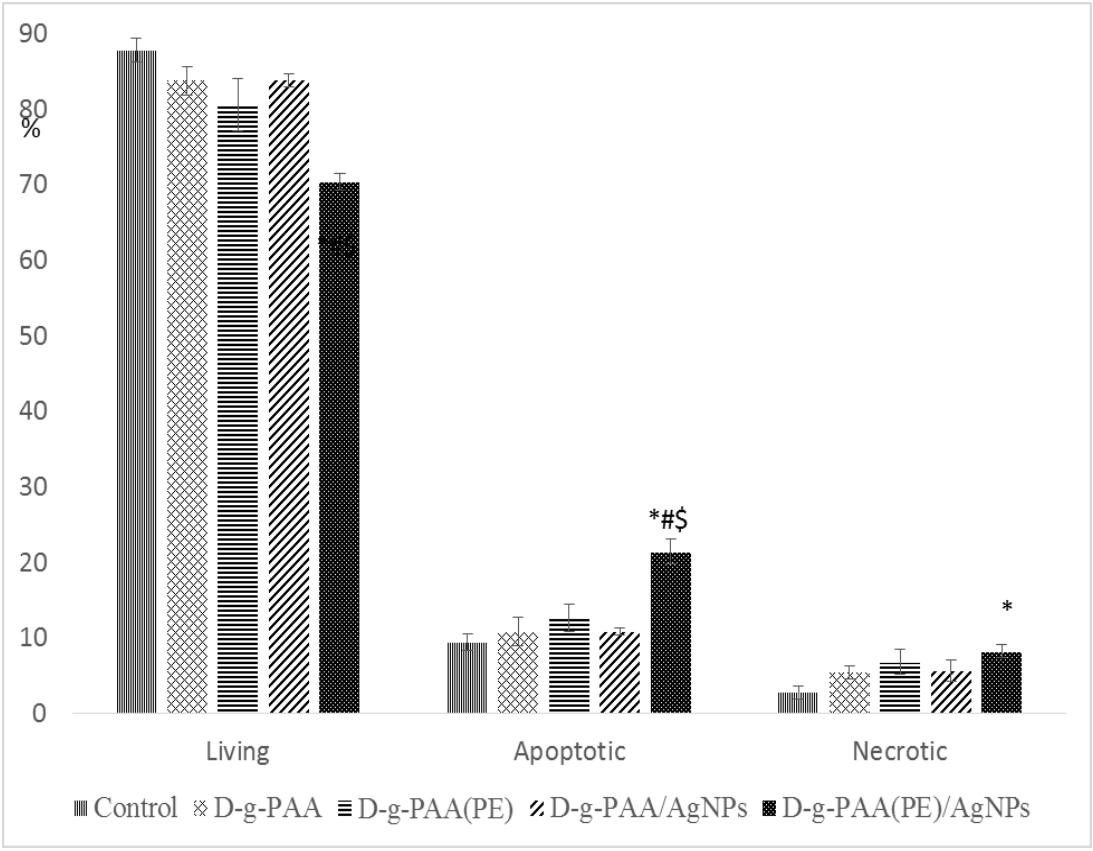

Fig. 1: The number of testicular cells with morphological signs of apoptosis and necrosis under conditions of treatment with silver nanoparticles in the polymer matrices.

Notes: * - p <0,05 - probability differences in the average group data with respect to these variables in the control group animals; # - p <0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA(PE); $ - p <0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA/AgNPs; M ± σ.

The number of living, apoptotic and necrotic spermatozoa (epididymis) sperm cells. Under conditions of treatment with both D-*g*-PAA and D-*g*-PAA(PE), no significant changes in the numbers of living, apoptotic and necrotic epididymal cells (spermatozoa) were observed (p>0,05, n=4) compared with the corresponding values in the control.

Under conditions of treatment with D-*g*-PAA/AgNPs, the number of living cells of the spermatozoa decreased (p<0,05, n=4), and the number of apoptotic increased (p <0,05, n=4), but no significant changes in the numbers of necrotic epididymis cells (spermatozoa) were registered (P>0,05, n=4) in comparison with the corresponding values in control.

Under conditions of treatment with D-*g*-PAA(PE)/AgNPs, the number of living cells of the spermatozoa decreased (p<0,05, n=4), and the number of apoptotic and necrotic increased (p <0,05, n=4), compared to the corresponding values in the control.

Under conditions of treatment with D-*g*-PAA(PE)/AgNPs the number of living cells of the epididymis (spermatozoa) decreased (p<0,05, n=4) and the number of apoptotic and necrotic increased (p<0,05, n=4) compared to the corresponding values under conditions of treatment with D-*g*-PAA(PE) and compared with the corresponding values under conditions of treatment with D-*g*-PAA/AgNPs.

Thus, under conditions of treatment with D-*g*-PAA(PE)/AgNPs, the number of living cells decreases and the number of cells with morphological signs of apoptotic and necrotic cell death in the epididymis (spermatozoa) increases (Fig. 2).

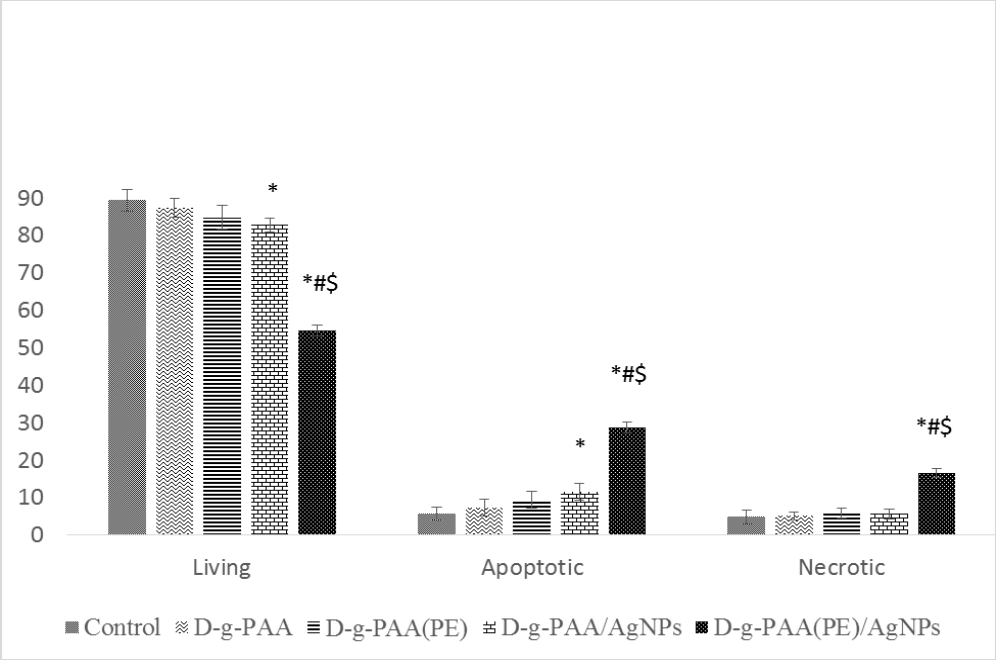

Fig. 2: The number of epididymis cells with morphological signs of apoptosis and necrosis under conditions of treatment with silver nanoparticles in the polymer matrices.

Notes: * - p<0,05 - probability differences in the average group data with respect to these variables in the control group animals; # - p<0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA(PE); $ - p<0,05 - to these variables in the group animals under conditions of treatment with D-*g*-PAA/AgNPs; M ± σ.

DISCUSSION

The potential pharmaceutical application of nanoparticles has led to the toxicity within the male reproductive system. Nanoparticles are generally able to reach the testicle in small quantities where they persist for several months, regardless of the route of exposure.[11]

Silver nanoparticles (Ag NPs) are one of the most explored nanostructures and are extensively used in various application. There is a recent review presenting literature data collected at various biological levels related to AgNP-induced reproductive toxicity.[12]

A good review of the literature on reproductive and developmental toxicity of AgNPs has also been previously published.[13]

By oral treatment. A high silver content in the testes was established after 120 days of oral administration at the the dose of 50 µg/day/animal of silver nanoparticles with an average size of 34 ± 5 nm stabilized with polyvinylpyrrolidone, and a significant accumulation of silver in the lungs and brain was observed.[14]

By sub-dermally treatment. Thay suggest that Ag-NP triggered hormonal imbalance and induce oxidative stress in testis and epididymis; which negatively affect sperm parameters of male rats. A total of 30 rats were divided into 6 groups and were sub-dermally exposed to Ag-NPs at the dosage of 0 (control), 10, and 50 mg/kg bodyweight (bw) doses for either 7 or 28 days.[15]

By intra-abdominal treatment. The effect of intra-abdominal injection of AgNP at different concentrations and different length of time on the testes in rats was studied.[16,17]

There are data on a significant decrease in the number of spermatogonia, Sertoli and Leydig cells, however, sperm chromatin evaluation showed no significant differences between the experimental groups.[17]

By intravenous treatment. Also, the effect of intravenous single dose silver nanoparticles (AgNP) on rat spermatogenesis was studied. They found dependence on size (20 nm and 200 nm), dose (5 and 10 mg/kg body weight) and time (24 h, 7 and 28 days) reduction in the number of epididymis sperm measured by histological methods. The treatment with AgNPs increased the level of DNA damage in germ cells, as measured by alkaline comet assay. 20 nm AgNPs were more toxic than 200 nm.[18]

By intratesticular injection. It has been evaluated the effects of an intratesticular injection of silver nanoparticles (AgNPs) on reproductive parameters and health of rats. Treated animals received 220 µL of AgNPs solution (0.46 µg-Ag/ml) in each testicle and were euthanized: seven, 14, 28, and 56 days after injection. A significant decrease ($p < 0.05$) in the percentage of motile sperm in D7 (8.8%) was observed, comparing to the control (73.3%), D14 (86.0%), D28 (68.2%), and D56 (90.0%) groups. D7 group also

presented a decrease ($p < 0.05$) in the percentage of normal.[19]

Today, the use of nanotechnology to create new highly efficient biomedical nanocomposites is one of the most relevant research topics. Nanocarriers based on the branched star-like copolymers (D-*g*-PAA and D-*g*-PAA(PE)) were synthesized, characterized and tested on phagocytic cells. It has been shown that these nanocarriers are actively captured by phagocytic cells and that they are not cytotoxic.[3]

Previously, we obtained data on the effect of D-*g*-PAA polymers on male and female reproductive function in mice.[5-7]

In this work under conditions of treatment with D-*g*-PAA(PE)/AgNPs it was shown: 1) a decrease in 1,70 times the number of live newborns (pups) per female; 2) an increase in 3,79 times preimplantation and in 2,93 times postimplantation embryonic mortality rates; 3) an increase in 1,92 times the number of abnormal forms of sperm (%) - an increase in the number of sperm with primary anomalies (heads) and secondary anomalies (tail) was found; 4) a decrease in 1,93 times the number of spermatocytes (primary) and in 2,01 times the spermatids (%); 5) a decrease in 1,25 times the number of living cells and an increase in 2,27 times the number of cells with morphological signs of apoptotic and in 2,93 times the number of cells with necrotic death in the testis (spermatocytes (primary)); 6) a decrease in 1.64 times the number of living cells, an increase in 4,96 times the number of cells with morphological signs of apoptotic and in 3,46 times the number of cells with necrotic death in the epididymis (sperm) - compared to the values in the control.

And compared to the values for D-*g*-PAA/AgNPs: 1) a decrease in 1,47 times the number of live newborns (pups) per female; 2) an increase in 3,15 times preimplantation - and 2,03 times postimplantation embryonic mortality rates; 3) an increase in 1,51 times the number of abnormal forms of sperm (%) - an increase in the number of sperm with primary anomalies (heads) and secondary anomalies (tail) was found; 4) a decrease in 1,81 times the number of spermatocytes (primary) and in 1,97 times the spermatids (%); 5) a decrease in 1,19 times the number of living cells and an increase in 1,98 times the number of cells with morphological signs of apoptotic and in 1,44 times the number of cells with necrotic death in the testis (spermatocytes (primary)); 6) a decrease in 1,52 times the number of living cells, an increase in 2,48 times the number of cells with morphological signs of apoptotic and in 2,96 times the number of cells with necrotic death in the epididymis (sperm).

The obtained data indicate a disorder of the reproductive function of males under conditions of treatment with silver nanoparticles in the polymer matrix D-*g*-PAA(PE). It is that polymer matrix D-*g*-PAA(PE) has such a particularly toxic effect on spermatogenesis and in which silver nanoparticles, in particular, less than 4 nm (2-5 nm in size), are placed. It is that size of the nanosilver that may be responsible for the effect, however, this needs to be studied in detail.

The data obtained agree that silver nanoparticles may be toxic to the reproductive system.[19-21]

For the first time it was shown, that under conditions of five times treatment with D-*g*-PAA(PE)/AgNPs (silver nanoparticles in the polymeric matrix D-*g*-PAA(PE)) were found the disorder of reproductive function in male mice: significant changes in the viability and death of spermatozoa and of spermatocytes (primary), as well as an increase in pre- and post-implantation embryonic mortality rates.

Our data suggest that treatment with such silver nanosystems (silver nanoparticles in the polymer matrix D-*g*-PAA(PE)) are not critically dangerous for therapeutic use and requires further study.

Further studies could be aimed at determining the biodistribution-accumulation-retention-removal of such copolymers. Particular attention should be paid to the interaction of the copolymers (and silver nanoparticles) with the immune system, as this is likely to determine certain nonspecific immune responses and the delivery of engineered particles and drugs to target organs, tissues or cells.

CONCLUSION

Under conditions of treatment with D-*g*-PAA and D-*g*-PAA(PE), as well as D-*g*-PAA/AgNPs, no significant changes in the values of 1) the number of live newborns (pups) per female; 2) pre- and post-implantation mortality rates of embryos were established; 3) sperm and abnormal sperm forms (%); 4) spermatocytes (primary) and spermatids (%); 5) living, apoptotic and necrotic cells in the testicular cells (spermatocytes (primary)) and in the epididymis cells (spermatozoa).

The data obtained indicate that branched star-like polymers in anionic form with incorporated silver nanoparticles (D-*g*-PAA(PE)/AgNPs) stimulate spermatogenesis impairments in testis and epididymis of male mice.

Our data suggest that treatment with such silver nanosystems (silver nanoparticles in the polymer matrix D-*g*-PAA(PE)) are not critically dangerous for therapeutic use and requires further study.

DECLARATIONS

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and Bogomoletz National Medical University (Kyiv, Ukraine) for conducting research on the functioning of the male reproductive system under various experimental conditions.

Conflict of interest: The authors declare that there is no conflict of interest.

Ethical approval: The study was approved by Bogomoletz Instytute of Physiology Ethical Committee, Kyiv, Ukraine.

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