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Original Research Article

Effect of gold nanocomposites treatment on male reproductive function under conditions of experimental hyperglycemia

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ABSTRACT

Background: Given the urgency of the problem of clinical and experimental pathology of diabetes mellitus (DM) and the reproductive function disorders and their correction, the direction of research was determined in the following way: to establish the functional state of testicular cells and appendages under experimental hyperglycemia, and to assess future prospects for gold nanoparticles for clinical trials. The aims to evaluate the effect of five times treatment with gold nanocomposites (gold nanoparticles (AuNPs) in the polymer matrix D-g-PAA(PE) on male reproductive function under conditions of experimental hyperglycemia.
Methods: Experiments (two series) have been conducted on 120 (60 males and 60 females) Albino white laboratory mice (weighing 25-30 gm). Experimental hyperglycemia, a model of type 2 diabetes mellitus (DM2) was reproduced by administration of nicotinamide and streptozotocin (internally peritoneally). The treatment of substances was carried out in the following way: D-g-PAA (PE) (10.00 mg/kg), D-g-PAA (PE)/AuNPs (9.78 mg/kg) in the tail vein, in 0.3 ml, once a day, five times, 2 weeks after EG induction once a day, five times.
Results: it was established for the first time that there is disorder of male reproductive function under conditions of experimental hyperglycemia and five-fold treatment of gold nanocomposites (D-g-PAA(PE)/AuNPs, namely an increase in the number of abnormal sperm and a decrease in spermatids, as well as an increase in preimplantation mortality of embryos (compared to this value under experimental hyperglycemia).
Conclusions: Our new data suggest that treatment with such gold nanocomposites (gold nanoparticles in D-g-PAA (PE) polymer matrix) are not critical for therapeutic use (in photodynamic chemotherapy), even in hyperglycemia when there is diabetes.

Keywords: Polymer matrix, Gold nanoparticles, Male reproductive function, Testis, Epididymis, Experimental hyperglycemia

INTRODUCTION

Nowadays, metal nanoparticles, in particular gold (nanoparticles of gold, AuNPs), have become widely used in the cosmetic, food and pharmacological industries. In scientific literature data on the ability of AuNPs to cross the hematotesticular barrier and cause disruption of the morpho-functional status of testicular cells are contradictory.[1-4]

Today, one of the most common diseases is DM. The World Health Organization estimates that more than 180

million people worldwide suffer from this pathology, and that number is expected to rise to 380 million by 2025.[5,6]

DM2 is characterized by hyperglycemia syndrome caused by impaired insulin action and/or secretion. High hyperglycemia and glycemic variability, as well as poor glycemic control are associated with patient disability and premature death as well as with the reproductive function disorders.[5-11]

The aims to evaluate the effect of five times treatment with gold nanocomposites (gold nanoparticles (AuNPs) in the polymer matrix D-g-PAA(PE) on mice male reproductive function under conditions of experimental hyperglycemia (EG), namely: 1) the amount of sperm (sperm concentration (millions/ml)) and the number of abnormal sperm (%); 2) the ratio of cells of different generations of spermatogenic epithelium (%) in the testes; 3) the number of living, apoptotic and necrotic cells of the testis (primary spermatocytes) and the number of living, apoptotic and necrotic cells of epididymis sperm; and provided that such males are mated with intact females for evaluation to evaluate: 1) the level of embryonic mortality before and after implantation; 2) the number of the live newborns (pups).

METHODS

The work was performed in the department of immune-physiology of the O.O. Bogomoletz institute of physiology, national academy of sciences of Ukraine in 2021 as part of scientific work No. III-15-20 "research of cellular and molecular mechanisms of immune-induced disorders of the female reproductive system and the corrective effect of metal nanoparticles"/state registration number of the work 0119U103964 (departmental).

Synthesis and characterization of polymer/AuNPs nano-system

According to the TEM data, the synthesized polymer/AuNPs systems contain AuNPs 2-11 nm in size and have a spherical shape. Details of synthesis, identification and analysis of gold nanoparticles have been described in literature.[12-17]

Experimental hyperglycemia (EG)

A model of type 2 DM was reproduced by administration of 120 mg/kg nicotinamide (internally peritoneally), then after 15 min, 70 mg/kg streptozotocin (internally peritoneally) once a day with an interval of one day (introduction of substances three times).

Assessment of the condition of animals

The blood glucose content of mice was measured 14 hours (overnight) after the last feeding (by depreciation of food). The level of glucose in the blood, which was taken by capillary from the tail vein in mice, was determined using diagnostic test strips for glucose determination (Citolab G, Pharmasco, Kyiv) (Ukraine).

Testicles were collected two weeks after the induction of experimental DM in mice with hyperglycemia greater than 14.0 mmol/L (14.7 mmol/L to 19.3 mmol/L). In intact (control) mice, the blood glucose level was 5.80±0.27 mmol/L; in experimental (after administration of nicotinamide and streptozotocin) blood glucose concentration increased in 2.9 times (17.20±0.39 mmol/L; $p<0,01$),

Animals

Experiments (two series) have been conducted on 120 (60 males and 60 females) Albino white laboratory mice (weighing 25-30 gm) in compliance with all requirements for work with laboratory animals (International European convention for the protection of vertebrate animals, Strasbourg, 1986). After the experiments, the animals anesthetized by Nembutal were exterminated by cutting the spinal cord.

In the first series of experiments

Animals (males) were divided into groups treated with: I-physiological solution-control (n=5); II - D-g-PAA(PE) (n=5); III-D-g-PAA(PE)/AuNPs (n=5); IV-EG (n=5); V-EG+D-g-PAA(PE) (n=5); VI-EG+D-g-PAA(PE)/AuNPs (n=5); n is the number of animals in the group. On the third day after the last (fifth) injection of substances under ether anesthesia, the experimental material (testes and epididymis) was collected. The animals were removed from the experiment by cutting the spinal cord under anesthetic anesthesia, following the rules of the euthanasia.

In the second series of experiments

Animals were divided in to the same groups treated with: I-physiological solution-control (n=5); II-D-g-PAA(PE) (n=5); III-D-g-PAA(PE)/AuNPs (n=5); IV-EG (n=5); V-EG+D-g-PAA(PE) (n=5); VI-EG+D-g-PAA(PE)/AuNPs (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's method (1990). Sampling of experimental material (ovaries, tubes, and uterus) was performed under anesthesia by Nembutal 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).

The treatment of substances was carried out in the following way

D-g-PAA(PE) (10.00 mg/kg), D-g-PAA(PE)/AuNPs (9.78 mg/kg), saline solution was introduced intravenously (in

the tail vein, in 0.3 mL) once a day, five times, two weeks after EG induction once a day, five times.

Sperm viability

To assess the viability of sperm, a dye test of trypan blue was performed. Viable cells remained unstained, whereas non-viable cells were stained blue. The ratio of viable cells to the total number of cells counted was calculated as a percentage of viability for each sample.

Estimation of the number of sperm (concentration of sperm (millions/ml)) and the number of abnormal forms of sperm (%)

Sperm derived from the caudal part of the epididymis were examined for morphology, and the number of sperm (the concentration of sperm (millions\ml)) and the number of abnormal forms of sperm (%). Sperm divided into 3 groups: normal (without deformation of structural elements of cells), primary and secondary anomalies. Sperm anomalies evaluated according to classifications of a number of authors and summarized by us (Table 1).

Table 1: Anomalies of sperm.

Norms	Anomalies	
	Primary	**Secondary**
Acrosome	Shortened\ absence of acrosome\ deformed	-
Head	Deformed	Duplicated\ No head
Neck	Spiral twisted	Bend
Tail	-	Curved shortened\ loop at the end\ no tail\ spiral twisted
All sperm	Numerous anomalies\ spiral\ ball	-
Sperm don't fuse\ separated from each other	Sperm merger	-

Evaluation of the ratio of cells of different generations of spermatogenic epithelium (%)

Three different populations of germ cells can be identified in suspensions of testis cells: spermatogonia, spermatocytes (primary and secondary) and spermatids. *Spermatogonia* (stem cells) are large round (spherical) cells, with a homogeneous cytoplasm and a well-expressed nucleus (type-A spermatogonia) do not contain heterochromatin in nuclei, but type-B have a large proportion of DNA in the condensed state, and also differentiate. Spermatocytes-primary are oval cells, the largest cells of the familial epithelium, tetraploid cells and recognized as having large nuclei; spermatocytes-secondary: oval but smaller cells. Spermatids are small spherical cells with a spherical nucleus and condensed heterochromatin without nuclei. Sertoli cells (supporting cells) have a triangular (pyramidal) shape. Their nuclei are irregularly shaped with invaginations, a "three-membered" nucleus (nucleus and two groups of nucleolus chromatin).

Method of color fluorescent dyes

The apoptotic and necrotic death of testicular cells (primary spermatocytes) and cells of the epididymis (sperm) was estimated by morphological characteristics using the method of *in vitro* dual-color fluorescent dye nucleic acids Hoechst 33342 and propidium iodide. At least 400 cells evaluated using a fluorescence microscope LUAMAM I-1 (Russia) with ×85 water-immersion lens.

Embryonic mortality in mice

The pre- and post-implantation death rates were calculated by formulas: ((C-A+B)/C)×100% and (B/(A+B))×100%, respectively. Counted: A is the number of live embryos, B is the number of sites of resorption (the number of dead embryos), C is number of yellow bodies of pregnancy.

Statistical analysis

For the statistical analysis of the results the software package Graph Pad prism version 5.01 (GPW5-930421-RAG-1368) for windows (Graph Pad Software, San Diego California, USA) has been used. The verification of the received data on normality of distribution was carried out on the Kolmogorov-Smirnov test. For a normal distribution, the statistical processing of the results when comparing the two data groups was performed using Student's t test, with more data groups using a single-factor ANOVA analysis, followed by a comparison of mean values between the groups according to the Newman-Keuls test; $p<0.05$ was considered statistically significant.

RESULTS

Number of sperm (concentration of sperm (millions/ml)) and the number of abnormal forms of sperm (%)

In comparison with such values in control the following data have been registered: 1) under the conditions of treatment with D-g-PAA(PE) no significant changes in the number of sperm, as well as the number of abnormal sperm and those with primary abnormalities (%); 2) under the conditions of treatment with D-g-PAA(PE)/AuNPs an increase in the number of abnormal sperm and those with primary anomalies (%); 3) under the conditions of EG a decrease in the number of sperm, as well as an increase in the number of abnormal sperm and those with primary anomalies (%); 4) under the conditions of EG and the introduction of D-g-PAA(PE) found a decrease in the number of sperm, as well as an increase in the number of abnormal sperm and those with primary abnormalities (%); 5) under the conditions of EG and treatment with D-

g-PAA(PE)/AuNPs found a decrease in the number of sperm, as well as an increase in the number of abnormal sperm and those with primary anomalies (%); 6) also under the conditions of treatment with D-g-PAA(PE)/AuNPs an increase in the number of abnormal sperm (%) compared to value under conditions of treatment with D-g-PAA(PE); 7) under the conditions of EG and treatment with D-g-PAA(PE) there is an increase in number of abnormal sperm and those with primary anomalies (%) compared to following values under conditions of treatment with D-g-PAA(PE) has been observed/ registered (Table 2).

The ratio of cells of different generations of spermatogenic epithelium (%) in the testes

In comparison with the following values in controlno significant changes in the number of spermatogenic cells and Sertoli cells on smears of homogenate of the testes under conditions of treatment with D-g-PAA(PE) were observed, while in other experimental groups a decrease in the number of spermatids (%) was registered. In addition, under conditions of EG and treatment with D-g-PAA(PE) a decrease in the number of spermatids (%) compared with this value under conditions of treatment with D-g-PAA(PE) was observed (Table 3).

Number of living, apoptotic and necrotic cells of the testis (spermatocytes (primary)

In comparison with such values in control the following data have been registered: 1) under the conditions of treatment with D-g-PAA(PE) no probable changes in the number of living, apoptotic and necrotic testicular cells (primary spermatocytes); 2) under the conditions of treatment withD-g-PAA/AuNPs a decrease in the number of living cells and an increase in necrotic testicular cells (primary spermatocytes); 3) under the conditions of EG there is a decrease in the number of living cells and an increase in the number of apoptotic and necrotic cells of the testis (primary spermatocytes); 4) under the conditions of EGand treatment with D-g-PAA(PE) decreases the number of living cells and increases the number of apoptotic and necrotic testicular cells (primary spermatocytes); 5) under the conditions of EGand treatment with D-g-PAA/AuNPs the number of living cells decreases and number of apoptotic and necrotic testicular cells (primary spermatocytes) increases (Table 4).

Number of living, apoptotic and necrotic epididymis spermatozoa

In comparison with the values in control the following results have been obtained: 1) under the conditions of treatment with D-g-PAA(PE) there is no probable change in the number of living, apoptotic and necrotic cells of the epididymis (sperm); 2) under the conditions of treatment with D-g-PAA/AuNPs a decrease in the number of living cells and an increase in the number of necrotic cells of the epididymis (sperm) is observed; 3) under the conditions of EG there is a decrease in the number of living and an increase in apoptotic and necrotic cells of the epididymis (sperm); 4) under the conditions of EG and treatment with D-g-PAA the number of living decreases and the number of apoptotic and necrotic cells of the epididymis (sperm) increases; 5) under the conditions of EG and treatment with D-g-PAA(PE)/AuNPs decreases the number of living and increases the number of apoptotic and necrotic cells of the epididymis (sperm) (Table 5).

The pre- and post-implantation death rates

Therefore, no probable changes were investigated in the values of pre- and postimplantation embryonic mortality under conditions of treatment with D-g-PAA(PE), while under conditions of treatment with D-g-PAA/AuNPs an increase in the values of preimplantation and postimplantation mortality of embryos in comparison with such values in the control has been registered, as well as in comparison with such values under conditions of treatment with D-g-PAA(PE). Under conditions of EG an increase in the values of pre- and postimplantation mortality of embryos in comparison with such values in the control has been established. Under conditions of EG and treatment with D-g-PAA(PE), there were no probable changes in the values of embryo mortality-pre-and post-in comparison with such values in the control. Under conditions of EG and treatment with D-g-PAA/AuNPs an increase in the values of preimplantation and postimplantation mortality of embryos has been established in comparison with such values in the control, as well as an increase in the value of preimplantation mortality of embryos in comparison with this value under conditions of EG (Table 6).

Table 2: Functional state of sperm under conditions of EG and treatment with gold nanocomposites.

Variables	The concentration of sperm, (1×106/ml)	Number of abnormal sperm (%)	
		Total	Primary anomalies
Control	36.40±1.29	17.60±1.70	5.80±0.50
D-g-PAA(PE)	35.40±0.95	21.40±1.63	7.80±0.95
D-g-PAA(PE)/AuNPs	35.20±0.81	30.80±1.89* #	9.20±0.95*
EG	28.60±0.81*	37.40±1.70*	13.20±0.95*
EG+D-g-PAA(PE)	28.20±1.29*#	44.60±1.71*#	17.80±1.41*#
EG+D-g-PAA(PE)/AuNPs	25.60±1.70*&	49.60±1.29*&$	22.60±1.29*&$

*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 D-g-PAA(PE) treatment; &p<0.05-to these variables in the group animals under conditions of D-g-PAA(PE)/AuNPs treatment; $p<0.05-to these variables in the group animals under conditions of EG; M±σ.

Table 3: The number (%) of testicular spermatogenic cells under conditions of EG and treatment with gold nanocomposites.

Variables	Number of Percentages (%)			
	Spermatogonia	Spermatocytes	Spermatides	Sertoli
Control	10.20±1.25	16.20±1.29	53.80±2.58	4.20±0.50
D-g-PAA(PE)	9.40±0.95	15.40±1.29	51.40±1.50	4.60±0.57
D-g-PAA(PE)/AuNPs	9.80±0.95	13.80±0.81	37,60±1.70*	4.60±0.57
EG	8.20±0.81	12.40±0.5	37.80±1.66*	4.80±0.81
EG+D-g-PAA(PE)	7.60±1.25	12.20±0.81	37.20±1.29*#	4.40±0.50
EG+D-g-PAA(PE)/AuNPs	7.20±0.57	10.20±0.5	28.40±0.81*&$	5.00±0.81

*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 D-g-PAA(PE) treatment; &p<0.05-to these variables in the group animals under conditions of D-g-PAA(PE)/AuNPs treatment; $-p<0.05-to these variables in the group animals under conditions of EG; M±σ.

Table 4: The number of testicular cells (spermatocytes (primary) with morphological signs of apoptosis and necrosis under conditions of EG and treatment with gold nanocomposites.

Variables	Number of spermatocytes (primary), (%)		
	Living	Apoptotic	Necrotic
Control	87.80±1.60	9.40±1.14	2.80±0.84
D-g-PAA(PE)	80.60±3.40	12.60±1.82	6.08±1.64
D-g-PAA(PE)/AuNPs	77.80±2.40*	15.80±1.92	6.40±0.89*
EG	69.80±0.80*	16.80±1.48*	13.40±1.95*
EG+D-g-PAA(PE)	68.40±1.70*#	18.4±2.51*	13.20±1.92*#
EG+D-g-PAA(PE)/AuNPs	61.60±1.70*&	21.40±1.52*	17.00±1.87*&

*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 D-g-PAA(PE) treatment; &p<0.05-to these variables in the group animals under conditions of D-g-PAA/AuNPs treatment; M±σ.

Table 5: The number of epididymis spermatozoa with morphological signs of apoptosis and necrosis under conditions of EG and treatment with gold nanocomposites.

Variables	Number of spermatozoa, %		
	Living	Apoptotic	Necrotic
Control	89.4±2.88	5.8±1.64	4.8±1.92
D-g-PAA(PE)	84.80±3.19	9.40±2.30	5.80±1.30
D-g-PAA(PE)/AuNPs	69.60±3.36*#	16.60±2.07*	13.80±1.48*#
EG	75.00±1.60*	14.20±1.48*	10.80±1.64*
EG+D-g-PAA(PE)	74.00±1.60*#	14.00±0.71*	12.00±1.58*#
EG+D-g-PAA(PE)/AuNPs	67.80±2.6*	18.20±1.92*	14.20±0.84*

*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 D-g-PAA(PE) treatment; M±σ.

Table 6: Embryonic mortality under conditions of EG and treatment with gold nanocomposites.

Variables	Preimplantation mortality	Post implantation mortality
Control	8.76±0.74	5.38±0.37
D-g-PAA(PE)	10.62±2.81	8.82±2.72
D-g-PAA(PE)/AuNPs	23.23±2.71*#	15.18±2.14*#
EG	16.35±2.54*	12.02±2.81*
EG+D-g-PAA(PE)	13.02±2.51	9.38±2.01
EG+D-g-PAA(PE)/AuNPs	25.54±2.31*$	16.62±2.81*

*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 D-g-PAA(PE) treatment; $-p<0.05-to these variables in the group animals under conditions of EG; M±σ.

Table 7: The number of live newborns (pups) under conditions of EG and treatment with gold nanocomposites.

Variables	Number of live new-borns
Control	7.25±0.95
D-g-PAA(PE)	5.75±0.50
D-g-PAA(PE)/AuNPs	4.50±0.57*
EG	4.25±0.50*
EG+D-g-PAA(PE)	5.25±0.64
EG+D-g-PAA(PE)/AuNPs	4.00±0.812*

*p<0.05-probability differences in the average group data with respect to these variables in the control group animals; M±σ.

Number of live newborns (pups)

Thus, there were no significant changes in the number of live newborns (pups) under conditions of treatment with D-g-PAA(PE), while under conditions of treatment with D-g-PAA/AuNPs a decrease in the number of live newborns (pups) compared with this value in the control was observed. A decrease in the number of live fetuses under conditions of EG was established compared with this value in the control. Under conditions of EG and treatment with D-g-PAA(PE) there were no significant changes in the number of live newborns (pups) compared with this value in the control. Under conditions of EG and treatment with D-g-PAA/AuNPs, there was established a decrease in the number of live newborns (pups) compared with this value in the control (Table 7).

DISCUSSION

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.[18] It has been shown that polymers with a dextran core and grafted polyacrylamide chains dextran-polyacrylamide (D-PAA) are effective in photodynamic chemotherapy, which gives confidence in the future of the drug nanosystem.[18,19]

Influence of gold nanocomposites

In our experiment we used the following dose of D-g-PAA(PE) polymers: 10.00 mg/kg (10 μg/g animal). It was assumed that a dose of 10 mg/kg body weight in mice is equivalent to a human dose of 0.81 mg/kg body weight, equivalent to approximately 50 mg for a human 60 kg, in accordance with the principles of conversion of animal doses to humans.[20]

And, following this rule, 10.00 mg/kg D-g-PAA(PE) in mice is equivalent to a human dose of 0.81 mg/kg body weight, which is equivalent to approximately 50 mg (48.6 mg) for human 60 kg (or 56.7 mg for humans 70 kg) in accordance with the principles of conversion of animal doses for humans; 9,78 mg/kg - D-g-PAA(PE)/AuNPs-55.45 mg/70 kg. Thus, a single dose of D-g-PAA(PE)/AuNPs (a total of five animals were administered according to the experimental scheme) is twenty times higher than the minimum dose, which is not expected to cause for a short period of a week, while in this work-five days marked toxicity and is at the maximum edge of the recommended range for similar studies in animals. Under the conditions of treatment of D-g-PAA(PE) in comparison with the following values in the control we did not find probable changes in the number of: 1) sperm, as well as the number of abnormal sperm and those with primary anomalies (%); 2) spermatogenic cells and Sertoli cells on smears of testicular homogenate; 3) living and dead by apoptosis and necrosis of testicular cells (spermatocytes (primary)) and cells of the epididymis (sperm); 4) and in the values of pre- and post-implantation embryonic mortality; 5) the number of live newborns (pups).

Whereas under the conditions of treatment of D-g-PAA(PE)/AuNPs in comparison with such values in the control no probable changes in the number of sperm were established; what has been registered is: 1) an increase of 1.75 times in the number of abnormal sperm and 1.59 times in those with primary abnormalities (%); 2) reduction of 1.43 times in the number of spermatids; 3) a 1.13 times decrease in the number of living testicular cells (spermatocytes (primary)) and a 2.86 times increase in the number of such necrotic cells; 4) a 1.28 times decrease in the number of living cells of the epididymis (sperm), and a 2.89 times increase in the number of such necrotic cells; 5) an increase of 2.65 times in the values of preimplantation and 2,82 times in post implantation mortality of embryos; 6) reduction of 1.61 times in the number of live newborns (pups).

Under the conditions of treatment of D-g-PAA(PE)/AuNPs in comparison with the conditions of treatment of D-g-PAA(PE) there has been established: 1) an increase of 1,44 times in the number of abnormal sperm; 2) a decrease of 1.22 times in the number of living cells of the epididymis (sperm) and a 2.4 times increase in the number of necrotic cells; 3) increase of 2.19 times in the value of preimplantation and 1.72 times in the value of postimplantation mortality of embryos.

The effect of five-time treatment with gold nanocomposites under conditions of experimental hyperglycemia (EG)

According to modern ideas, two mechanisms play a key role in the pathogenesis of DM: 1) violation of insulin secretion by P cells; 2) increased peripheral resistance to

insulin (decreased peripheral glucose uptake by the liver or increased glucose production). In recent decades, numerous models of experimental diabetes have been developed, the main ones being chemical, surgical, endocrine, immune and genetic. Chemical models of DM, which can be induced by chemicals (alloxan, streptozotocin, dithizone, etc.), which selectively affect the P cells of the islets of Langerhans, have been widely used.[21-23]

Known that streptozotocin diabetes is modeled by the introduction into animals of a synthetic drug streptozotocin, which can selectively penetrate into the P cells of the pancreas using the vector GLUT-2. The mechanism of action of streptozotocin is due to the alkylating activity of its methyl group, which inhibits DNA synthesis, which leads to P cell death, is enhanced by the activation of free radical oxidation associated with the generation of peroxynitrite formed from excess nitric oxide, the source of which is nitric nitric oxide.[24]

The study of diabetes as a free radical pathology began relatively recently, but, a single model for assessing the role of oxidative stress in the pathogenesis of diabetes and analysis of the effectiveness of antioxidant therapy does not yet exist.[23-28]

In our experiment, under conditions of EG we have established in comparison with the following values in the control: 1) a decrease of 1.27 times the number of sperm; 2) an increase of 2.13 times in the number of abnormal sperm and 2.28 times in those with primary abnormalities; 3) a 1.42 times reduction in the number of spermatids in the testes; 3) a 1.26 times decrease in the number of living testicular cells (sperm cells (primary)), and a 1.79 times increase in the number of apoptotic and 4.76 times necrotic cells; 4) a 1.19 times decrease in the number of living cells of the epididymis (sperm) and a 2.45 times increase in the number of apoptotic and 2.25 times necrotic cells; 5) an increase of 1.87 times in the value of preimplantation and 2.23 times postimplantation mortality of embryos; 6) reduction of 1.71 times in the number of live fruits.

These data have been obtained using animals in which the blood glucose level (after administration of nicotinamide and streptozotocin) under EG conditions increased in 2.9 times (17.2±0.39 mmol/L; $p<0.01$) compared with this value in animals of the control group (5.80±0.27 mmol/L).

Thus, we obtained new data on the disorder of the male reproductive function under conditions of EG.

Under the conditions of EG and the treatment of D-g-PAA(PE)/AuNPs in comparison with this value in the control we have registered: 1) a decrease of 1.42 times in the number of sperm; 2) an increase of 2.82 times in the number of abnormal sperm and 3.89 times in those with primary abnormalities; 3) reduction of 1.9 times in the number of spermatids; 4) a decrease of 1.42 times in the number of living testicular cells (spermatocytes (primary)) and a 2.28-fold increase in the number of apoptotic and 6.07 times in necrotic cells; 5) decrease in 1.32 times in the number of living cells of the epididymis (sperm) and 3.14 times increase in the number of apoptotic and 2.96 times in necrotic cells; 6) an increase of 2.92 times in the value of preimplantation and 3,09 times the value of postimplantation mortality of embryos; 7) a decrease of 1.81 times in the number of live newborns ($p<0.05$, $n=4$).

It is important to note that under the conditions of EG and the introduction of D-g-PAA(PE)/AuNPs in comparison with this value under the conditions of EG the following data have been obtained: 1) an increase of 1.33 times in the number of abnormal sperm and 1.71 times in those with primary abnormalities (%); 2) reduction of 1.33 times in the number of spermatids; 3) a 1.56 times increase in the value of preimplantation mortality of embryos.

The data presented here expand and supplement the data on the effect of branched polymers and gold nano-systems on the female and male reproductive system, obtained by us earlier.[29-32]

In our experiments in adult mice, it was shown that the introduction of gold nanoparticles in the polymer matrix D-g-PAA(PE) leads to impaired reproductive function in male mice under EG conditions. There is interesting evidence that an increase in the number of sperm with abnormal heads may lead to an increase in post implantation losses, preimplantation death may increase in accordance with a decrease in the fertility of sperm not associated with genetic disorders (tail abnormalities).[33]

Our data suggest that treatment with such nano systems of gold (gold nanoparticles in the polymer matrix D-g-PAA(PE)) are not critically dangerous for therapeutic use, even on an initial stage of chronic kidney disease, when there is already kidney damage, which is accompanied by impaired filtration and manifested by proteinuria (the appearance of protein in the urine). However, further studies are needed to clarify the dose (to reduce), multiplicity (increase and decrease) and the way of treatment with such gold nano systems.

In the future, we will investigate the following questions: 1) how the nanoparticles of gold D-g-PAA(PE)/AuNPs (spherical shape, size 2-11), loaded (synthesized, retained) in D-g-PAA(PE) made an impact (influenced), while matrices such as D-g-PAA(PE) had no such influence? 2) how and where exactly in the body such gold particles are released from the matrix (and whether they are released at all!)? 3) what other structures of the organism can lead to such an outcome, if it is not a direct effect on germ cells? Since at present these questions remain unanswered, further studies are needed. conducted under the conditions of *in vitro*, further research may to advance our understanding of the processes taking place.

Alternatively, it would be reasonable to investigate the interaction of such matrices with blood, blood plasma,

proteins, and blood form elements, especially with erythrocytes or platelets. Also, special attention should be paid to the interaction of copolymers (nanoparticles) with the immune system, as this is likely to determine certain non-specific immune responses and delivery of engineered particles and drugs to target organs, tissue or cells.

CONCLUSIONS

It was established for the first time that there is disorder of male reproductive function under conditions of experimental hyperglycemia and five-fold treatment of gold nanocomposites (D-g-PAA(PE)/AuNPs, namely an increase in the number of abnormal sperm and a decrease in spermatids, as well as an increase in preimplantation mortality of embryos (compared to this value under experimental hyperglycemia). Our new data suggest that treatment with such gold nanocomposites (gold nanoparticles in D-g-PAA(PE) polymer matrix) are not critical for therapeutic use (in photodynamic chemotherapy), even in hyperglycemia when diabetes is present.

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