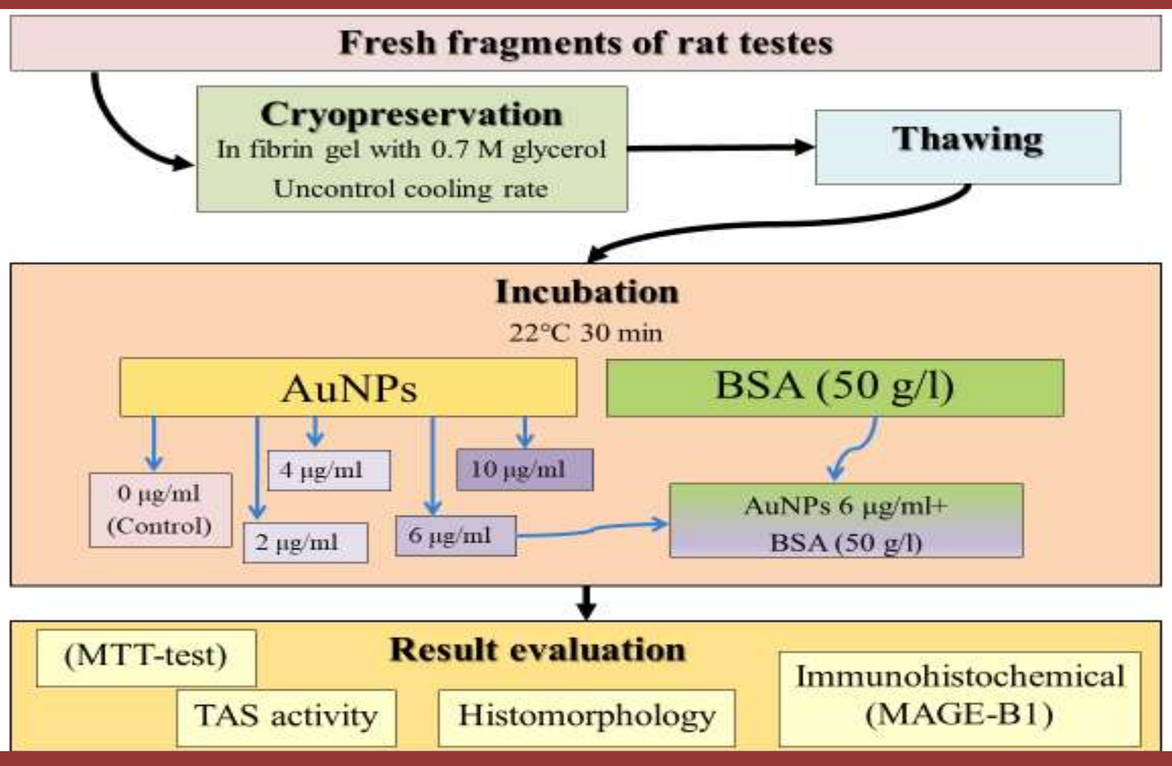
Effect of nanobiopolymers on morphofunctional state of cryopreserved testicular tissue

N.O. Volkova, M.S. Yukhta, L.V. Sokil, L.G. Chernyshenko, L.V. Stepanyuk, A.M. Goltsev
Institute for Problems of Cryobiology and Cryomedicine, Natl. Acad. of Sci. of Ukraine, Kharkov
e-mail: volkovana781@gmail.com

Introduction

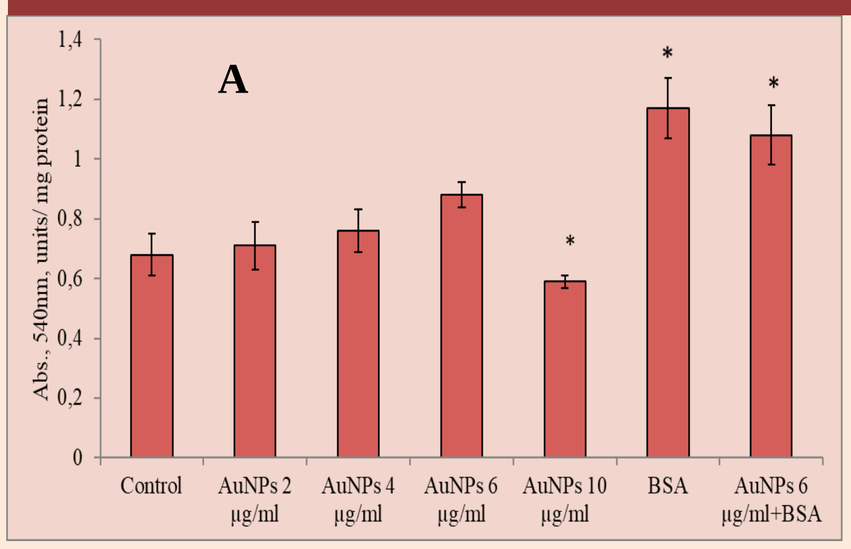
The gold nanoparticles (AuNPs) are very attractive for usage in biomedical technologies due to their unique properties and conventional methods of synthesis. These nanoscale metal can have different impact on both physical and chemical properties of cells, depending on their quantity or therapeutic dose. Now cryopreservation of testis tissue is a promising approach to save fertility in prepubertal boys undergoing gonadotoxic therapies. The using of nanobiopolymers as a basis of rehabilitation medium can be effective for the increasing of morphofunctional state of cryopreserved immature testicular tissue. This study represents a comparative evaluation of the effect of biopolymers (bovine serum albumin) in combination with gold nanoparticles (AuNPs) on morphofunctional characteristics of cryopreserved seminiferous tubules.

Materials and Methods



The AuNPs (Sigma-Aldrich, USA) with average size 15 nm were obtained by citrate synthesis with an initial metal concentration of 45 µg/ml. The tissue fragments after cryopreservation-thawing were incubated in Leibovitz's medium supplemented with AuNPs in concentrations of 2, 4, 6 or 10 µg/ml, bovine serum albumin (BSA) in concentrations of 50 g/l and combination BSA with AuNPs 6 µg/ml. Histomorphometric data, MTT-test and total antioxidant status (TAS) was determined in the samples after 30 min incubation. TAS activity was estimated quantitatively by the method of UV spectrophotometry (ERBA CHEM 7) using test kits (Randox, UK) according to the manufacturer's instructions and normalized to 1 mg of protein. Tissue fragments incubated under the same conditions without AuNPs and BSA were taken as a control. The results were processed with Student's t-test using Excel software.

Results



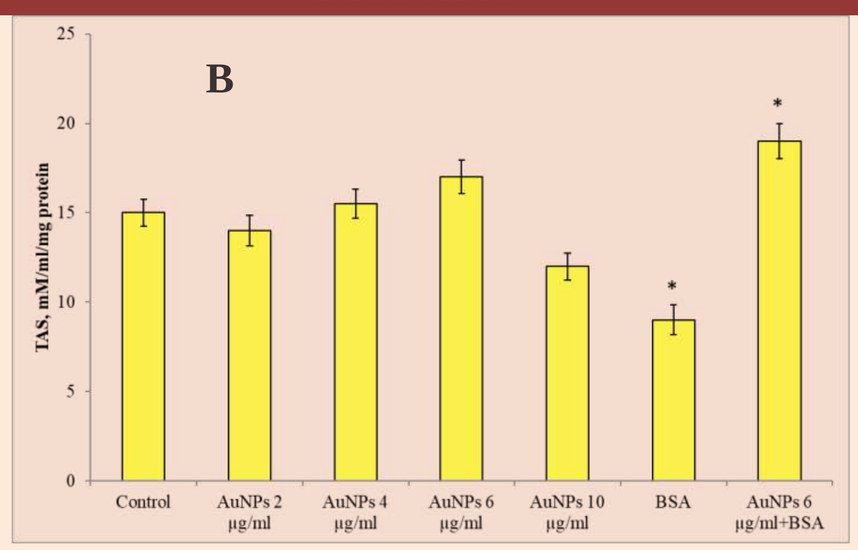


Fig.1 Effect of rehabilitation of cryopreserved seminiferous tubules in the media with AuNPs and BSA on:
A – metabolic activity (MTT test);
B – TAS activity.
* – the difference is statistically significant relative to the control ($p<0.05$)

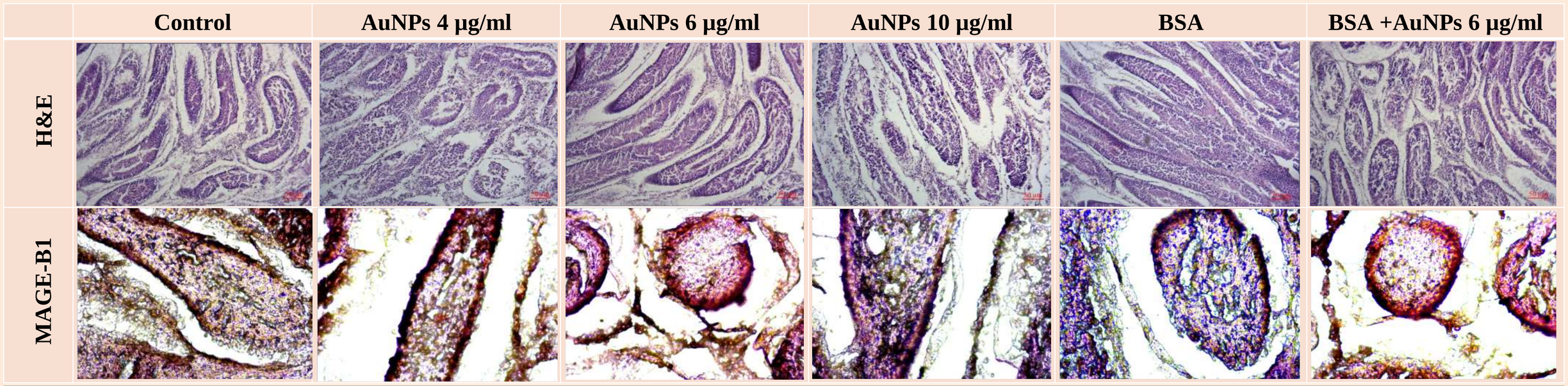


Fig.2 Cryopreserved seminiferous tubules of immature rat testes after rehabilitation in media with AuNPs and BSA. Staining with hematoxylin and eosin and immunohistochemical staining for MAGE-B1 (brown color), x400.

Conclusions

Our results demonstrated that the use of the low concentration of AuNPs (2-6 µg/ml) did not lead to significant changes in the studied indexes. The AuNPs application in the concentrations of 10 µg/ml resulted in a decrease of TAS and metabolic activity as well as in an increase of the number of necrotic cells in histological samples compared with the control. The AuNPs in investigated concentrations did not effect the relative number of MAGE-B1+ cells in seminiferous tubules of testes. The analysis of TAS activity, MTT-test and histomorphological data showed that the combination of the BSA 50 g/l with AuNPs 6 µg/ml was the most optimal for rehabilitation of cryopreserved seminiferous tubules combining the positive properties of both substances. The obtained data can be used for substantiation and development of effective rehabilitation methods for cryopreserved seminiferous tubules using the combination of biopolymers and nanoparticels.