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MAIN ASPECTS TRANSFER FOREIGN GENES IN THE GENOME OF SPERM JAPANESE QUAIL (*COTURNIX JAPONICA*)

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This paper describes a process of transferring genetic construction (pEGFP-N1 vector) into the genome of Japanese quail sperm using dimethyl sulfoxide (DMSO). Transfer and expression of the introduced genetic information was evaluated by the characteristic fluorescence of transfected spermatozoa due to expression of the green fluorescent protein (GFP) and by PCR analysis.

Japanese quail, sperm, genetic constructions, ejaculate, transfection.

In recent years, developed a number of methods for transgenic animals. methods consist of: microinjection into pronuclear, somatic cell nuclear transfer and transduction using retroviruses. Last most widely used, however, retroviral method has several disadvantages, such as restrictions on the size of the built-gene and the dangers of using this technique. An alternative and inexpensive method is the introduction of foreign DNA in the sperm to fertilize. Although transgenic animals were obtained from the use of sperm-mediated gene transfer (sperm- mediated gene transfer (SMGT)), though the effectiveness of this technique was Lo-tuple that was caused poor penetration of exogenous DNA in sperm. The lack of SMGT effectiveness was associated with activation of protective mechanisms of sperm and seminal plasma, resulting in degradation of exogenous DNA took place.
The purpose of research - an increase ektyvnosti transfection of sperm from the use of dimethyl sulfoxide (DMSO) and composites for washing of DNA-polymerase.

Materials and methods research. To determine the effect of seminal plasma on the intensity of incorporation of exogenous DNA in sperm samples were combined and washed with 109 sperm vidmyvayuchym solution tsentryfuhuyuchy at 600 g six times. After each wash, the supernatant was removed for testing DNKaznu activity, which was measured by horizontal gel electrophoresis (1% agarose -a) using imaging

DNA 0.5 mg / ml ethidium bromide. Mobility and sperm activity was assessed by phase-contrast microscopy before and after each washing.

Exogenous DNA adsorbovuvalas on the surface of sperm treatment removed 20 U DNA-polymerase I (Invitrogen, USA) for 30 min and three washes in phosphate-buffered saline (PBS) at 600g for 5 minutes.

Isolated from sperm DNA was analyzed by PCR for detection of internalization (learning) of exogenous DNA.

Conclusions

Thus, the use of ejaculate of DNA-polymerase and subsequent transfection using DMSO enable effective mobile and get transfected fertile sperm.

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