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Гормональний статус старих щурів з аліментарним ожирінням після введення пуповинної крові

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Hormonal Status of Aged Rats with Diet-Induced Obesity After Umbilical Cord Blood Administration

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The number of obese people steadily increases (Acosta A, 2014). Disorders of central mechanisms of regulation, when the eating behavior changes and body's neurohormonal shifts occur, are observed in obesity (Hesketh K, 2018). The objective herein was to evaluate the impact of administration of cryopreserved nucleated cord blood cells (cNCBCs) on hormonal status of aged rats with obesity. Experiments were approved by the Bioethics Committee of the Institute for Problems of Cryobiology and Cryomedicine of the NAS of Ukraine. Thawed CBNCs (Mancinelli F, 20219) product was injected intraperitoneally in dose of 3×10^5 $CD34^+$-cells/kg. The product was made up to 1 ml volume with plasma autologous to the cells. The next day, a week and a month after cord blood injection, the animals were sacrificed and blood was sampled for further studies. The content of thyroxine (T4), triiodothyronine (T3), testosterone (Ts), estradiol (ES) and dehydroepiandrosterone sulfate (DHEAS) was determined by enzyme-linked immunosorbent assay using the standard ELISA kits. The obesity was accompanied by a significant decrease in thyroid and sex hormone concentration in blood serum of aged rats. The cNCBCs administration the next day, a week and a month later promoted increasing not only the total T3 level, but also total T4 one in blood serum of aged obese rats, accompanied by a significant increase in Ts concentration as well. The cNCBCs (Kramer J., 2020) injection augmented the functional activity of thyroid and sex glands (Cooper D., 2015), normalized the Ts level, thus increasing the adaptive and compensatory body potential in aged obese animals. The revealed changes are physiologically significant, since thyroid hormones increase the oxygen absorption by organs and tissues, regulate the metabolism of lipids and carbohydrates, thereby participating in energy metabolism and maintaining the constancy of body weight. If a metabolic rate increases, the proteins and fats are decomposed under thyroid hormone impact, resulting in decreased appetite and body weight loss.

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Оптимізація протоколу кріоконсервування мезенхімальних стовбурових клітин пуповини людини

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Optimization of Human Umbilical Cord Mesenchymal Stem Cell Cryopreservation Protocol

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Cell transplantation is a promising method of treating various human diseases. Mesenchymal stem cells derived from human umbilical cord have a great potential for both fundamental research and clinical applications. Their value lays in their high proliferative ability, differentiation plasticity, phenotype stability, non-immunogenicity and immunomodulatory properties. The mesenchymal cells obtained from perinatal tissues do not contain major histocompatibility complex proteins, which prevents post-transplant rejection of these cells.

Cryopreservation is a reliable method that ensures the efficacy of preserving the properties and number of stem cells in the long term. The success of cryopreservation depends first of all on an optimally designed cell cryopreservation protocol.

The purpose of the study is a selection of optimal conditions for freezing and cryopreservation of stem cells from the human umbilical cord.

Cell suspension of 1 ml (2,000,000 cells, counted using a hemocytometer) diluted in conditioned medium DMEM/F12 Advanced was added to 2 ml cryotubes. Afterwards cryoprotective medium containing 60% fetal bovine serum (FBS), 30% conditioned medium, and 10% dimethyl sulphoxide (DMSO) were added in 2 stages.

To prevent cell damage and loss of functions a protocol for freezing the stem cells was created. Due to the mixing of the freezing medium with the cell suspension in equal proportions, the concentrations of FBS and DMSO were reduced by half to 30 and 5% respectively. To minimize the toxic effect of DMSO cryoprotectant on stem cells the freezing medium was divided into two equal portions and added in two stages. After the first stage the cells were kept at 4ºC for 15 min. After adding the second part of the freezing medium, cryovials were put in the programmed freezer where the temperature decreased gradually at a rate of 1ºC per minute. The percentage of viable cells after 4–5 years of liquid nitrogen storage (–196ºC) was 85–90%. Among the advantages of the optimized protocol we can highlight reducing of the cytotoxic cryoprotectant DMSO doses to 5% (compared to the commonly used 10%), the use of lower concentration of FBS (lowering the cost of the procedure), application of conditioned medium enriched with metabolites and secretions of stem cells (for creating favorable conditions for cells during freezing time), addition of the freezing medium in two stages in order to reduce its harmful effect.

The optimized cryopreservation protocol contributed to the achievement of high survival efficiency of stem cells after long-term liquid nitrogen storage, providing reliable and suitable conditions for cell culture preservation.