

‘Cold in Biology and Medicine: Current Problems in Cryobiology, Transplantology, and Biotechnology’ is the conference of young scientists, organized by the Council of Young Scientists mutually with administration of IPC&C and UNESCO Chair in Cryobiology. Conference is held annually in May.

Conference is organized to encourage the young scientists’ creative and intellectual initiative, shearing their research, making an insight for academic community into scientific activity of young scientists, making the contacts between scientists.

The participants of the conference can be young scientists, PhD- and university students.

The working languages of the conference are Ukrainian, Russian and English.

The conference covers the investigations devoted to various aspects of low temperature effect on biologicals:

- mechanisms of damage to biological objects under the influence of low and extremely low temperatures;
- low temperature storage of biological objects (human, animal or plant cells, tissues, organs), including preparation for storage, thawing;
- cultivation of cells and tissues, etc.
- the response of biological objects to low temperature;
- the mechanisms of natural resistance of living systems to the cold and the process of recovery from cold exposure
- cold adaptation of animals and plants;
- low temperature in medicine;
- experimental and clinical transplantation;
- cryobiological and cryomedical devices;
- low temperature banks and depositories *etc.*

Search for methodological approaches of organotypic cultivation for following cryopreservation

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Introduction. A number of modern pharmaceuticals and transplantation methods require the use of small organs and tissues fragments with the preserved interactions of cells. These can be organotypic cultures, cell-engineering structures, 3D prints. Cryopreservation of such objects greatly expands the possibilities of their application. When working with multicellular structures, it is necessary to overcome specific problems, such as the phenomena of central necrosis, infiltration with a cryoprotectant, and features of crystal formation.

The aim of the research was to find methods of organotypic cultivation and evaluation of mouse organ slices for following cryopreservation.

Materials and methods. The organs of BALB/c mice were used. Using a vibratome, sections (slices) were made from animal organs embedded in agarose blocks. The possibility of using various concentrations of agarose in different organs was determined: liver, brain, spleen, heart, kidney, testicles, ovaries, lungs, and uterus. Light microscopy and screening methods for evaluating objects were used.

Results and discussion. A necessary step in the manufacture of slices is pouring in agarose from 3 to 5% at a temperature of not more than 45°C, which allows for making slices thickness from 0.02 to 0.5 mm. The quality and thickness of the samples depend on the structure of the tissue, the number and density of connective tissue fibers, the size of the structural and functional elements. It is possible to obtain thin slices of nervous tissue – from 0.05 mm. Slices of the liver and kidneys should be made with a thickness of about 0.3 mm, which corresponds to the size of their structural and functional units (lobules and glomeruli, respectively). Activities with the lungs, spleen, and heart are complicated by the density of connective tissue and the lack of tight fixation in agarose. The production of thin slices of the testicles is not possible due to the slight connection between the tubules, which are separated from each other during the cutting stage. For screening evaluation of slices, trypan blue staining, the use of light microscopy, and tetrazolium and resazurin tests are possible.

Conclusions. The quality of the slices for organotypic cultivation and cryopreservation depends on the density of the connective tissue, the size and the relationship between the structural elements. It is possible to obtain high-quality slices of nervous tissue, liver, and kidneys.

Study of cryopreserved placental cells biological effect on organotypic uterine culture

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Many pathological conditions in obstetrics, gynecology and reproductology are caused by pathology of the uterus and endometrium. During pregnancy, the uterus is the organ that is most affected by humoral factors produced by the placenta. When studying the mechanism of action of placental derivatives on the female reproductive system, it is important to exclude the influence of the immune and endocrine systems, which becomes possible under the conditions of cultivation of organotypic uterine culture (OUC) in an *in vitro* system.

The aim of the work was to study the effect of media conditioned with native and cryopreserved placental cells on mouse organotypic uterine culture.

To obtain OUC, the uterus of BALB/c mice in the estrus phase were isolated, cut into fragments up to 3 mm, cultured in 24-well plates at a rate of 10 mg tissue/ml of DMEM enriched with 10% FBS (control) in a CO₂ incubator at 37°C in an atmosphere of 5% CO₂. To study the effect of placental derivatives, the cultivation of OUC was performed in conditioned DMEM medium (CM), in which placental cells (PC-CM) or cryopreserved placental cells (CPC-CM) were pre-cultured. The metabolic activity of the samples was assessed by the resazurin reduction test.

The primary uterine tissue fragments (UTF) had a typical structure: the inner layer of the endometrium consisted of large cylindrical cells with heterochromic nuclei; a large number of glands were observed between connective tissue cells, the muscle layer was well defined, tightly adhered to the serous and outer layers with blood vessels and elements of adipose tissue. During short-term cultivation of UTF in DMEM, the overall structure, size, and ratio of the mucous, muscle, and serous layers of tissue fragments were preserved. After cultivation of UTF in both PC-CM and CPC-CM their general structure was preserved with simultaneous hyperhydration of the tissue, and the absence of a clear distribution of endometrial and biometric layers. There were signs of destructuring in endometrial tissue and glands with the presence of isolated cells and detritus. The indices of metabolic activity of UTF after cultivation in both PC-CM and CPC-CM did not differ, however, they were significantly higher compared to control.

The data obtained showed that UTF cultivated with media conditioned with placental derivatives undergo more significant morphological changes compared to the control, and are characterized by a higher intensity of metabolic processes. The determined effect of placental derivatives on the OUC in the *in vitro* system is typical for the processes occurring in the uterus *in vivo* during pregnancy. The nature and degree of the effect of cryopreserved placental cells in the composition of CM on OUC correspond to the effect of native cells.