Probl Cryobiol Cryomed 2022; 32(4):307
https://doi.org/10.15407/cryo32.04.307

Пошук методологічних підходів органотипового культивування для подальшого кріоконсервування

А. Каверінська[1], А. Ткаченко[2], В. Прокопюк[1,2]
[1]Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна
[2]Харківський національний медичний університет, м. Харків, Україна

Search for Methodological Approaches of Organotypic Cultivation for Following Cryopreservation

A. Kaverinska[1], A. Tkachenko[2], V. Prokopiuk[1,2]
[1]Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine
[2]Kharkiv National Medical University, Kharkiv, Ukraine

A number of modern pharmaceuticals and transplantation methods require the use of small organs and tissue fragments with the preserved interactions of cells. These can be organotypic cultures, cell-engineering structures, and 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.

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 such as: liver, brain, spleen, heart, kidney, testicles, ovaries, lungs, and uterus was determined. Light microscopy and screening methods for evaluating objects were used.

A necessary step in slice manufacturing 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 fibres, 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.

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.

Probl Cryobiol Cryomed 2022; 32(4):307
https://doi.org/10.15407/cryo32.04.307a

Вивчення біологічного впливу кріоконсервованих клітин плаценти на органотипову культуру матки

Т. Михальчук, М. Шевченко, В. Прокопюк, О. Прокопюк
Інститут проблем кріобіології і кріомедицини НАН України, м. Харків, Україна

Study of Cryopreserved Placental Cells Biological Effect on Organotypic Uterine Culture

T. Mykhalchuk, M. Shevchenko, V. Prokopiuk, O. Prokopiuk
Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

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 uteri 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 CO_2 incubator at 37°C in an atmosphere of 5% CO_2. To study the effect of placental derivatives, the cultivation of OUC was performed in a 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 the 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 the control.

The data obtained showed that UTF cultivated with media conditioned with placental derivatives underwent more significant morphological changes compared to the control, and were 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.