

Probl Cryobiol Cryomed 2025; 35(4):240
<https://doi.org/10.15407/cryo35.04.240>

**Possible use of sucrose for pretreatment
and subsequent cryopreservation of newborn rats'
brain-derived neural cells**

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The use of neural cells (NCs) in regenerative medicine and biomedical research requires efficient and safe cryopreservation methods. Standard protocols with dimethyl sulphoxide (Me₂SO) and fetal bovine serum (FBS) have limitations due to the cryoprotectant toxicity and the presence of xenogenic components. Sucrose, as a non-toxic disaccharide, is considered a promising alternative for cryoprotection. The effectiveness of using sucrose for pretreatment and cryopreservation of MSCs has been shown previously (Petrenko Y., 2014). The aim of the study was to evaluate the possibility of using sucrose for pretreatment and subsequent cryopreservation of NCs isolated from the brain of newborn rats.

Primary neural cell suspension was isolated from newborn rats' brains (P0) by a combined enzymatic-mechanical method and cultured in 25 cm² flasks in DMEM F12 with 10% FBS. The majority of cells in the formed monolayer exhibited glial morphology, but cells with neuronal and neuroblast morphology were also observed. The primary NC culture was subcultivated and reseeded in 6-well plates in equal concentration. After plating, NCs were pretreated (PT) with 0.05, 0.1, 0.2 and 0.3 M sucrose solutions (based on DMEM/F12 with 10% FBS) during 24 hours. Then each group was cryopreserved (CP) in a range of sucrose solutions (0.05, 0.1, 0.2 and 0.3 M) without cryoprotectant and serum. The control group was not treated with sucrose and CP in DMEM/F12 containing 10% FBS and 10% Me₂SO. All groups were cryopreserved in cryovials at a cooling rate of 1 deg/min down to -80 °C followed by plunging into liquid nitrogen. Cryopreserved samples were thawed on water bath at 38 °C. After sucrose PT and CP, we assessed the cell viability by Trypan blue staining and the ability to attach and spread during cultivation.

Pretreatment with sucrose did not affect the viability of NCs, which averaged 96%. The viability of cryopreserved cells in the control group was 78%, whereas the viability of pretreated NCs depended on the combination of sucrose concentrations used. The best results in the NC samples were achieved with combinations of 0.1–0.2 M sucrose for pretreatment and 0.2–0.3 M for freezing (viability 56–58%). Cryopreserved NC on the 2nd day of cultivation formed a 70% monolayer in the control group. At the same time, in the pretreated/cryopreserved NCs, the highest percentage of flattening was observed in the groups with sucrose combinations of 0.2/0.2 M, 0.1/0.3 M and 0.2/0.3 M — 30, 40 and 42%, respectively. Morphologically, all cultures (control and experimental) were dominated by glial cells with the presence of neuroblast-like and neuronal cells. Our preliminary studies have shown that the use of sucrose for the cryopreservation of NCs can be considered as a promising approach for the cryopreservation of neural cells without xenogenic and toxic compounds.

Probl Cryobiol Cryomed 2025; 35(4):240
<https://doi.org/10.15407/cryo35.04.240a>

**The influence of the implant of cryopreserved
placenta tissue on morphofunctional state
of recipient's placenta**

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The use of a cryopreserved placental tissue implant (CPTI) has proven that during long-term storage, it does not change its phenotypic characteristics of mesenchymal stem cells capable of differentiation and migration of the damaged organ.

The purpose of the study was to investigate the morphological changes of the placenta of rats against the background of implantation of CPTI.

The research was carried out on 30 pregnant female Wistar white rats, which were implanted with a piece of CPTI (1/4 part of the disc) under the skin of the back in the period of 1 week of gestation. The placenta was obtained under aseptic conditions from the uterus of a healthy rat on the eve of delivery, perused, fragmented and frozen according to a special program. After implantation of CPTI on the 7th and 14th day, the animals were removed from the experiment and the implant was removed. A microscopic study was performed comparing CPTI of recipients at the end of pregnancy and healthy Wistar rats that did not receive CPTI. Microscopic preparations after paraffin embedding and hematoxylin-eosin staining were studied.

Seven days after implantation, segmental nuclear infiltration, which is a manifestation of karyorrhexis, is microscopically detected in a piece of CPTI. Fourteen days after CPTI implantation, the placental implant was completely lysed. In the spongy part, layers of deciduas and trophoblast cells of the syncytiotrophoblast were preserved, which looked not dead, despite the presence of segmental leukocyte infiltration in the stroma.

When comparing the microscopic picture of the fetal and maternal parts of the placenta in animals with CPTI implantation, an increase in the number of trophoblastic cells with a large dark nucleus is observed. This fact can be interpreted as slowing down the aging of the placental tissue and preserving its hormonal and other functions. In addition, the implantation of CPTI is accompanied by a sharp decrease in the number of fibrinoid foci and their volume. It is reasonable to assume that the implanted piece of CPTI is included in the general chain of hormonal regulation in the mother-placenta-fetus system. After a certain increase in the amount of steroid hormones in the body, there is a gradual decrease in their production in the implant and a decrease in their content in the blood, which can stimulate the effect on the hypothalamus-pituitary-ovary system of the female according to the principle of feedback.

Implantation of cryopreserved placental tissue has a positive effect on the morphofunctional state of the placenta of the female rat recipient in the form of an improvement in the quality of the morphological structure of the placental tissue with a slowdown in its aging at the end of the gestation period.