Cryopreservation of cell cultures derived from dorsal root ganglia (DRG) is relevant in biomedical research. These cultures are valuable model objects for studying the mechanisms of sensory perception, axonal transport and nerve regeneration. DRG contain multipotent neural crest derived stem cells, capable to differentiate into neurons and various subpopulations of glial cells [Singh RP. et al., 2009]. In previous study of cell culture [Ali C. et al., 2018] we obtained the spheroids (Sph) from neonatal piglets’ DRG, wherefrom after re-plating there was a migration of the cells of different morphological types. However, the question about belonging them either to the subpopulations of neurons or glial cells has remained unanswered.

The research object was to study the expression of β-III-tubulin, glutamin synthetase and S-100 with subculturing native and cryopreserved spheroids derived from the cells of neonatal porcine dorsal root ganglia.

The cell suspension obtained from DRG of the neonatal piglets by enzymatic method was plated at a concentration of 0.5 × 10⁴ cells/ml and cultured with alpha-MEM medium with 2% Neuromax at 37°C and 5% CO₂. The spheroids formed on day 8 were cryopreserved in the cryoprotective media based on alpha-MEM and 25% fetal bovine serum (FBS) containing cryoprotectant DMSO in the concentrations of 5%, 7.5% and 10%. Cryopreservation was performed by a three-stage freezing program with an initial cooling rate of 0.5 deg/min down to –20°C; then with cooling rate of 1 deg/min down to ~80°C followed by an immersion into liquid nitrogen. Native and cryopreserved Sph were subcultured with alpha-MEM medium enriched with 10% FBS. To day 10 the expression of β-III-tubulin, glutamin synthetase (GS) and S-100 was analyzed in native and cryopreserved cultures by means immunocytochemistry methods.

In the culture derived from native Sph, large, spreading multipolar cells (type 1), small spindle-like cells with two small processes (type 2), and round or pyramidal cells with long processes (type 3) were observed. No expression of S-100 was found in the culture derived from native Sph. Expression of GS (a marker of satellite glial cells) was detected in the cells of the 1st and 2nd types, and expression of β-III-tubulin (a marker of neuroblasts and neurons) was revealed in the cells of the 3rd type. All the types of cells described above as well as the characteristics of their staining with specific markers were present in the culture derived from cryopreserved Sph.

Thus, in the culture obtained with subculturing Sph, there were the cells expressing the neural markers GS and β-III-tubulin. Cryopreservation in the used regimen preserves these cell subpopulations.