

The world population is predicted to reach approximately 9.7 billion people in 2050, so the demand for food, including meat, will continue to increase, contrary to the ability to produce the resources necessary for its production. Conventional meat production is associated with a destabilizing effect on the environment due to that *in vitro* meat production (cultured meat) can be safer (pathogen-free), more environmentally friendly (through lower consumption of water resources and land) and healthier for consumers alternative to traditional husbandry. Using the ability of muscle stem cells to proliferate, differentiate and self-renew their population, it is possible to construct three-dimensional structures *in vitro* that correspond to muscle tissue.

The co-culture of satellite cells (muscle precursor cells) with fibroblasts (connective tissue cells) is an important aspect of *in vitro* meat production. Previous studies focusing on understanding the effect of fibroblasts on muscle cells have shown their stimulating effect on the course of the process of myogenesis - muscle formation and confirmed the important role in the regeneration of damaged muscle fibers. However, most of these studies have focused on cell-cell interactions and few have been concerned on paracrine signaling (where molecules released by cells act on nearby cells) - including extracellular vesicles that are important biomolecules for carrying genetic information, proteins, metabolites, or lipids to other cells. In addition, the inconsistency in the available literature regarding the importance of paracrine communication in the co-culture of muscle cells and fibroblasts requires research to clarify this connection.

The proposed project aims to understand the impact of paracrine signaling - extracellular vesicles, and specifically the subgroup of exosomes (the most homogeneous population among all extracellular vesicles) on changes at the molecular level of genes and signaling pathways of the myogenesis process. We expect a beneficial effect of fibroblast-derived extracellular vesicles on satellite cell proliferation and differentiation. Extracellular vesicles derived from *in vitro* cultures of fibroblasts will be isolated and characterized according to international standards, and then their effect on muscle cells will be studied in *in vitro* culture. For this purpose, the RNA sequencing technique will be used to study the amount and sequence of genetic material. In addition, proteomic analysis (analysis of proteome sequence) by Western Blot and immunofluorescence with antibodies for selected myogenesis proteins will be performed.

The proposed research will allow to determine the importance of paracrine communication in the form of fibroblast-derived extracellular vesicles to muscle cells to improve methods of obtaining meat by *in vitro* techniques. In addition, the proposed research results can be used in other fields, such as medicine.