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Interaction between amniotic stem cells and canine mammary tumor “in vitro” – partial results
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Tumors in mammary glands are the most common neoplasm in dogs. They are identified according to the histopathology and classified according to the tissues which originate. The treatment of breast cancer in dogs is based on surgery and adjuvant chemotherapy. Thus, the aim of this study is to evaluate the treatment of canine mammary tumor cells using stem cells obtained from canine amniotic membrane, chemotherapy and the association between them. Totally, 20 samples of breast carcinoma will be collected and 10 samples from healthy mammary gland (control) will be collected for establishing cell cultures. After that, cocultures will be established using tumor cells treated only with canine amniotic membrane, tumor cells treated with canine amniotic membrane associated with chemotherapy (doxorubicin), and tumor cells treated only with chemotherapy for 24, 48, and 72 h. At the end, will be evaluated the metabolic activity of the cells, cell proliferation, cell cycle phases, mitochondrial membrane potential, and expression of cell markers. The carcinoma samples were collected and washed in PBS with 5% streptomycin/penicillin. Then, in order to dissociate the cells, an enzymatic protocol was used. For that, the explants were placed in polypropylene conical tubes with enzymatic digestion medium (containing DMEM-high glucose and 0.1% type I collagenase) for two hours at 37°C. Thereafter, the digestion medium was centrifuged, the supernatant discarded and the pellet resuspended in culture media containing DMEM-high glucose, 10% FBS, 1% streptomycin/penicillin, and 1% non-essential amino acids. 24 hours after to be plated into the dishes, the first adhered cells were observed. The cells had a fibroblast-like morphology with elongated cytoplasm and central nucleus. The cells had a rapid growth capacity, typically observed for tumor cells. After 2-3 days of cultivation, the cells reached 80% of confluence, it being possible to promote the expansion by trypsinization. It is expected that this research will establish a new treatment using amniotic stem cells associated or not with doxorubicin in order to treat canine mammary tumor.