IN VITRO ANGIOGENIC STIMULATION OF HUMAN OVARIAN TISSUE WITH SMALL EXTRACELLULAR VESICLES

Ana Sofia Pais, Miguel Lino**,** Sandra Reis, Alexandra Carvalho, Mafalda Laranjo, Ana Paula Sousa, Fátima Silva, João Ramalho-Santos, Filomena Boteho, Lino Ferreira, Teresa Almeida-Santos.

Reproductive Medicine Unit, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal;University of Coimbra, Faculty of Medicine, Obstetrics Department, Coimbra, Portugal;University of Coimbra, Institute of Biophysics and Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal;University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal;Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal;CNC - Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal, University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal; CNC - Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal., CNC - Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal; PhD Programme in Experimental Biology and Biomedicine, IIIUC – Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal., Reproductive Medicine Unit, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal; CNC - Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal; CICS-UBI-Health Sciences Research Centre, University of Bei, University of Coimbra, Institute of Biophysics and Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal; University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal;, Reproductive Medicine Unit, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal; CNC - Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal., Pathology Unit, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal., CNC - Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal; University of Coimbra, Department of Life Sciences, Coimbra, Portugal;, University of Coimbra, Institute of Biophysics and Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal; University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal;, University of Coimbra, Center for Innovative Biomedicine and Biotechnology (CIBB), Coimbra, Portugal; CNC - Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal; University of Coimbra, Faculty of Medicine, Coimbra, Port, Reproductive Medicine Unit, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal; CNC - Center for Neuroscience and Cell Biology, CIBB, University of Coimbra, Coimbra, Portugal; University of Coimbra, Faculty of Medicine, Coimbra, Portug.

Abstract Body

INTRODUCTION: Exosomes or small extracellular vesicles (SEVs) contain a mixture of biomolecules, with an important role in cell/tissue communication. SEVs seem to have a therapeutic effect in premature ovarian insufficiency (POI), by repressing apoptosis of granulosa cells and promoting angiogenesis. Iatrogenic POI, due to gonadotoxic treatments, can be overcome with ovarian tissue (OT) cryopreservation and transplantation. However, graft survival and lifespan are limited by the initial ischemia. Therefore, maintenance of follicular and tissue viability during the freezing/warming process and after transplantation is a major challenge.

AIM OF THE STUDY: To evaluate if SEVs are able to improve microvessel density in human cryopreserved OT.

MATERIAL AND METHODS: SEVs were isolated from human umbilical cord blood mononuclear cells. Frozen/thawed human OT from 7 patients was cultured for 48 hours in an alginate scaffold and divided into 4 groups: control (with no supplementation), VEGF 0.1 μg/mL with FGF 0.15 μg/mL and two different SEVs concentrations (1 μg/mL and 3 μg/mL). Internalization of SEVs in OT was observed by immunofluorescence. Follicular analysis was conducted after haematoxylin and eosin staining. Apoptosis,

cell proliferation and microvessel density were assessed by immunohistochemistry. Cytotoxicity was evaluated using lactate dehydrogenase (LDH) levels in culture medium.

RESULTS: The immunofluorescence assay allowed the identification of the SEVs in the human OT. Microvessel density was higher in tissue exposed to the 3 μg/mL concentration of SEVs as compared to control and VEGF+FGF groups (P=0.037 and P=0.02, respectively). There were no differences in follicular density, as well as in cell proliferation and apoptosis both in follicles and stroma. Cytotoxicity after 48h hours of culture, was similar in all culture conditions.

CONCLUSION: SEVs internalization in the cryopreserved human OT was associated with increased microvessel density and no impact on tissue viability, namely on follicular density, cell proliferation, and apoptosis of the tissue. Thus, the use of exosomes may be a therapeutic strategy to improve angiogenesis and reduce ischemia/reperfusion injury with transplantation.