Secretome by Human Mesenchymal Stromal Cells can Mitigate Age-related Ovarian Dysfunction by acting on both Follicle and Stroma Compartments: focus on Signaling Pathways deregulated by released MiRNAs

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Natural ovarian aging is characterized by an ongoing decline in the primordial follicle reserve and an increase in the general inflammation of the ovarian stroma both in human and mouse (Camaioni *et al*., 2022). In particular, in women this decline is associated with reduced fertility and irregular menstruation that leads to menopause in the early fifties, and it is strongly associated with the onset and progression of several alterations, such as osteoporosis, cardiovascular disease and cognitive dysfunction.

The aim of the present study is to determine whether conditioned medium (CM) of human Mesenchymal Stromal Cells (MSCs) derived from Adipose Tissue (ADSCs) and Dental Pulp (DPSCs), generally waste material, is able to mitigate the ovarian aging in 129/Sv females. In more detail, we analyzed different components of the secretome to which this effect can be attributed. Among them, the microRNAs (miRs) highly (HE) and differentially (DE) expressed between CMs have been evaluated for their biological effects in the aged ovaries.

The protocol consisted in intravenous administration of 5 ml/Kg of CMs collected after 3 days of MSC culture in aged 129/Sv females (8 months), every other day for three times, with one repetition after four weeks. One month later, some females were sacrificed and ovaries collected to perform follicle count, morphological analysis and gene expression, while other females were mated to follow their reproductive output. The miRs present in CM-DPSC and CM-ADSC were analyzed using a commercial column purification kit and sequenced on the Illumina platform. After detecting the HE and DE miRs, the TargetScan web tool identified the binding sites of miRs to the UTR region of gene transcripts, while the Enrichr web tool found the pathways deregulated by them.

The analysis of histological sections showed that the treatment increased the number of total follicles, mainly primordial and primary follicles, compared to control group, increased the pregnancy rate, the average number of pups delivered per mating cycle and the duration of the entire reproductive life. We then performed a statistical analysis to detect the HE/DE miRs among the 3-day CMs that have been used in *in vivo* experiments and have showed the capacity to counteract ovarian aging. Interestingly, miRs with high expression in both CMs, and those most highly expressed in each of them, showed binding sites for genes involved in signaling pathways related to follicle ovarian reserve (FoxO, PI3K-Akt), follicular atresia (apoptosis, P53), and inflammatory processes (IL-2, TNF).

These data indicate that treatment with MSC-CMs may mitigate ovarian aging and improve the functional status of the entire reproductive system, probably acting on the inflammatory state of the ovarian stroma, on the follicle reserve and the oocyte development potential. The analysis of miRs secreted in CMs highlighted their fundamental role in counteracting degenerative processes associated with ovarian aging, but underlined also the possibility of using them as therapeutic agents for the menopause-associated dysfunctions that affect skeletal, cardiovascular and nervous systems.