**EVs From Adipose-derived Mesenchymal Stem Cells Can Be Identified In Matched Serum In Outbred Canines: Potential for Aging Biomarker Discovery**

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**Background and aims.** Aging causes disruptions in the adipose tissue stem cell niche that lead to obesity as well as other aging-related diseases. Companion dogs are an excellent model of aging with parallels to human disease conditions. Extracellular vesicles (EVs) are membraneous nanostructures produced naturally by all cells, including adipose-derived mesenchymal stem/stromal cells (ADMSCs). The molecular cargo contained within ADMSC-derived EVs has the potential to be used to detect disease at an early stage with high specificity and sensitivity (1) however, significant technical challenges in EV characterisation mean that identification of cell-specific EVs in serum is inherently difficult (2).

**This project aims** to develop EV-based diagnostic tests that reflect the adipose stem cell niche in order to identify novel serum biomarkers for early detection of age-associated diseases. To our knowledge, this is the first study to evaluate the characteristics of matched ADMSC-derived and serum-derived EVs from the same dogs, which improves identification accuracy.

**Methods.** ADMSCs from 8 out-bred, privately owned dogs (3 female, mean age 25 months, >10 kg) were isolated from fat tissue explanted during their desexing procedure and with informed consent from their owners. Serum from the same dogs was obtained from whole, clotted blood. ADMSCs were characterised as CD90+CD44+CD29+CD45-CD34- by flow cytometry and averaged 0.367% of the live stromal vascular fraction. Cells were expanded until passage 2 and were grown in DMEM media containing FBS. At 70% confluence, cells were triple washed and serum-free DMEM was added. After 2 days, media containing EVs was collected. EVs from ADMSCs and matched serum were isolated and characterised.

**Results.** EVs isolated using TFF had an average size of 120nm and zeta potential averaging -20mV. The concentration of EVs was 2x109 particles per mL. In addition, AFM studies confirmed spherical morphology of EVs.

**Conclusion/Discussion.** Non-invasive biomarkers are crucial for the longitudinal study of disease, especially those related to aging. Age-related changes in ADMSC function may be early indicators of disease and therefore ADMSC-derived EVs may contain critical reflections of the adipose stem cell niche.

**References**

1) Phan et al. (2022) J Cell Sci. 10.1242/jcs.259166

2) Turner. (2024) Proteomics. 10.1002/pmic.202400074