Dear Editor,

Thank you for the opportunity to review the manuscript for the journal PeerJ Life & Environment. I have included my comments below.

The manuscript titled "Proteomic Profiling of Highly Abundant Secreted Proteins from Human Amniotic Stem Cells" by Dan et al. focuses on characterizing secreted proteins from amniotic mesenchymal stem cells (AMSCs) and amniotic epithelial cells (AECs). The authors employed mass spectrometry-based proteomics to identify hub proteins and explore their associated biological functions. Their findings were supported by techniques including LC-MS/MS, quantitative PCR (qPCR), and bioinformatic analysis.

However, the rationale, design, and interpretation of the data have many significant weaknesses, and the manuscript requires many changes before it can be accepted for publication.

1. Molecular studies: The authors aim to identify highly abundant proteins in adipose-derived stem cells (ADSCs). They report that the secreted protein ANXA2 is highly expressed in their ADSCs and enhances the expression of inflammatory factors in macrophages. However, they did not employ additional molecular or histopathological techniques to confirm the protein expression of the cytokines beyond using only quantitative PCR (qPCR)?

2. The investigators' use of a fixed concentration of 10 ng of Annexin A2 in the treatment and time model does not effectively demonstrate the optimal effect of Annexin A2 in this study. To validate the molecular results, the investigators should optimize their approach by varying the model conditions.

3. The manuscript's English requires thorough revision to eliminate grammatical and typographical errors and overstatements.

4. ANXA2 was observed to enhance the expression of pro-inflammatory cytokines in macrophages, but the molecular mechanism was not clearly described in the discussion.

5. Some testing procedures are inadequately described in this manuscript. The authors should provide a detailed account of the testing methods, such as how peptide samples were analyzed using LC-MS/MS.

6. Some irrelevant references do not support this study (Lines# 52-61). The authors need to review their references and ensure their relevance to this study.

7. Replication. Although the investigators used an N = 3 in their groups to Validate the expression of inflammatory factors mRNA in macrophages, the "model" treatment was only done once. The readers have no idea of the variability when the "model" treatment is repeated.

8. The authors identified highly abundant secreted proteins, particularly FLNA, TAGLN2, and Col3a1; however, no molecular or pathological studies examining their pathophysiological functions were conducted.

9. Lines# 48-51 state that no abbreviations should be used in the text. Authors must include the correct abbreviations for scientific terms.

10. Lines# 39-41 this information is insufficient for the start of the introduction.