Single extracellular vesicle analysis in human amniotic fluid shows evidence of phenotype alterations in preeclampsia

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***Supplementary Table 1.*** *Volume of amniotic fluid and the corresponding concentration of EVs as particles/ml of initial volume, based on nanoparticle tracking analysis.*

|  |  |  |
| --- | --- | --- |
|  | Normal pregnancies | Preeclampsia |
| Sample N.  | Sample volume (ml) | Concentration (particles/ml) | Sample volume (ml) | Concentration(particles/ml) |
| 1 | 20 | - | 12 | 2.80E+09 |
| 2 | 10 | 4.5 E+09 | 46 | 3.48E+09  |
| 3 | 6 | 2.58E+09 | 11 | 1.13E+09 |
| 4 | 12 | 2.81E+09 | 15 | 2.21E+09 |
| 5 | 4 | 3.8E+09 | 15 | 1.03E+09 |
| 6 | 4 | 3.6E+09 | 15 | 5.02E+09 |
| 7 | 10 | 2.53E+09 | 15 | 3.30E+09 |
| 8 | 4 | 6.59E+08 |  |  |
| 9 | 19.5 | 2.75E+09 |  |  |
| 10 | 4.5 | 2.18E+09 |  |  |
| 11 | 22 | 3.18E+11 |  |  |
| 12 | 15 | - |  |  |
| 13 | 22 | 3.08E+09 |  |  |
| 14 | 21 | 2.61E+09 |  |  |
| 15 | 20 | 3.08E+09 |  |  |
| 16 | 10 | 4.5E+09 |  |  |
| 17 | 6 | 2.58E+09 |  |  |
| 18 | 12 | 2.81E+09 |  |  |
| 19 | 4 | 3.8E+09 |  |  |
| 20 | 4 | 3.6E+09 |  |  |
| 21 | 19 | 2.79E+09 |  |  |
| 22 | 15 | -  |  |  |
| 23 | 19 | 3.65E+09 |  |  |
| 24 | 22 | 2.27E+09 |  |  |
| 25 | 10 | 4.5E+09 |  |  |
| 26 | 6 | - |  |  |
| 27 | 30 | - |  |  |
| 28 | 15 | 2.27E+09 |  |  |
| 29 | 19 | 4.9E+08 |  |  |
| 30 | 19 | 2.98E+09 |  |  |
| 31 | 15 | 2.27E+09 |  |  |
| 32 | 19 | 2.81E+09 |  |  |
| 33 | 22 | - |  |  |
| 34 | 10 | - |  |  |
| 35 | 25 | 6.776E+10 |  |  |
| 36 | 18 | 5.481E+10 |  |  |
| 37 | 22.5 | - |  |  |
| 38 | 41 | 3.914E+11 |  |  |
| 39 | 27.5 | - |  |  |
| 40 | 15 | - |  |  |

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***Supplementary Figure 1****.* ***ExoView analysis of amniotic fluid-derived NP-EVs (n=3) and PE-EVs (n=3).*** *5.8x108 EVs in final volume of 35 µl of buffer were used for all samples.* ***A)*** *Image in panel B was created with* [*https://biorender.com*](https://biorender.com)*.* ***B)*** *ExoView chips spotted with single tetraspanins and detected with anti- CD63, CD81, CD9 fluorescent Abs confirmed a similar tetraspanins expression level between NP and PE-EVs. Three different samples were analysed for each experimental condition. No statistical differences were observed.*



***Supplementary Figure 2****. Western Blot image and band intensity quantification showing CD105 expression differences between NP-EVs and PE-EVs.* ***A****) Representative image of the Western Blot showing the presence of CD105 and CD63 in NP- and PE-EVs.* ***B)*** *Quantification of Western Blot. CD63 was used as a loading control and the total lane intensity of CD105 was normalized to CD63 and used for the protein analysis. Results of two different analyses, each using pools of three different NP-EV or PE-EV samples, are shown.*