

Reduced level of placental protein 13 (PP13) in syncytiotrophoblast extracellular vesicles (STB EVs) of preeclampsia patients at delivery

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Introduction:

Extracellular vesicles (EVs) are an important cell-derived component in communicating pathological conditions and pregnancy disorders including preeclampsia (PE) - one of the most life threatening pregnancy disorders. A hallmark of PE is elevated placental shedding of syncytiotrophoblast extracellular vesicles (STB-EV) into the maternal circulation. Previous studies have implicated modified STB-EVs shedding in expression in PE along with their included protein galectin 13 (gals13 or PP13) which is one of the putative placental biomarkers. Other studies have shown reduced PP13 RNA in PE in both the placenta and in maternal serum, and low level of PP13 at early gestation.

Methods:

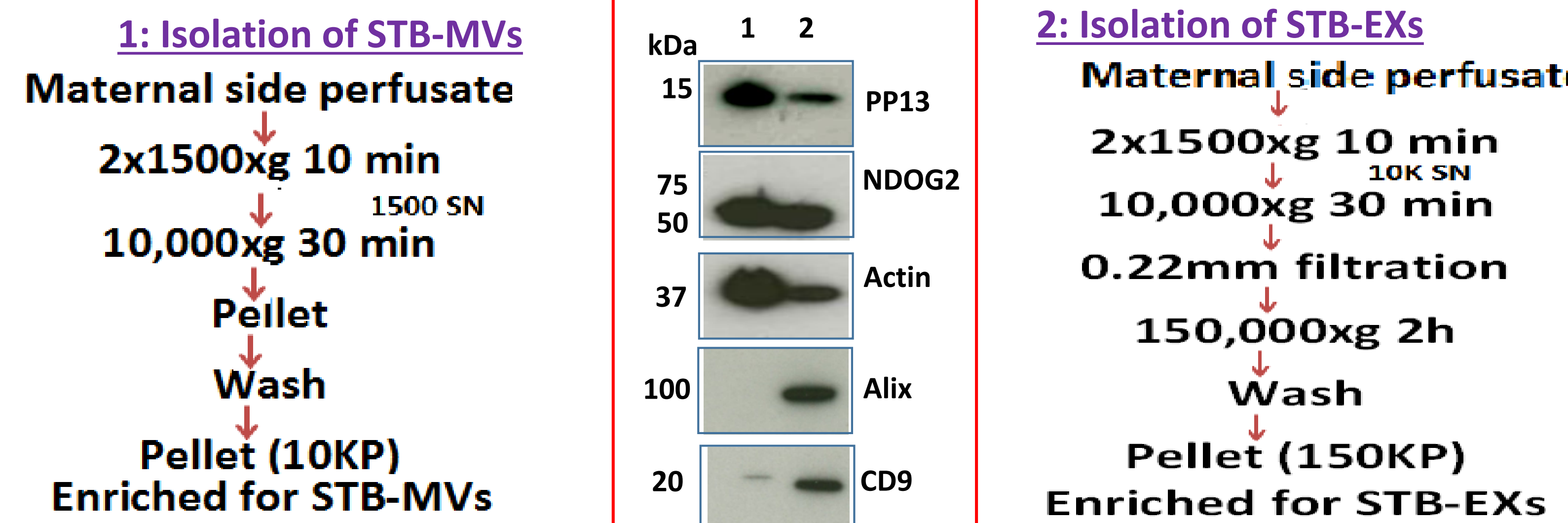
1. Placentae (n=20) were collected from women undergoing elective caesarean sections.
2. STBEVs were prepared using a dual placental lobe perfusion system following sequential centrifugation and filtration.
3. The purity and size distribution were assessed by Nanoparticle Tracking Analysis
4. Western blot analyses was used to verify the enrichment of the 10 KP (STB-MVs) and 150 KP (STB-EX) by their respective proteomic cargo.
5. PP13 expression was determined by Western blots and quantified by ELISA immunoassay

Aim of the study:

To explore the differences in STB-EV PP13 levels in PE compared to normal placentae collected by perfusion from the dual placental lobes.

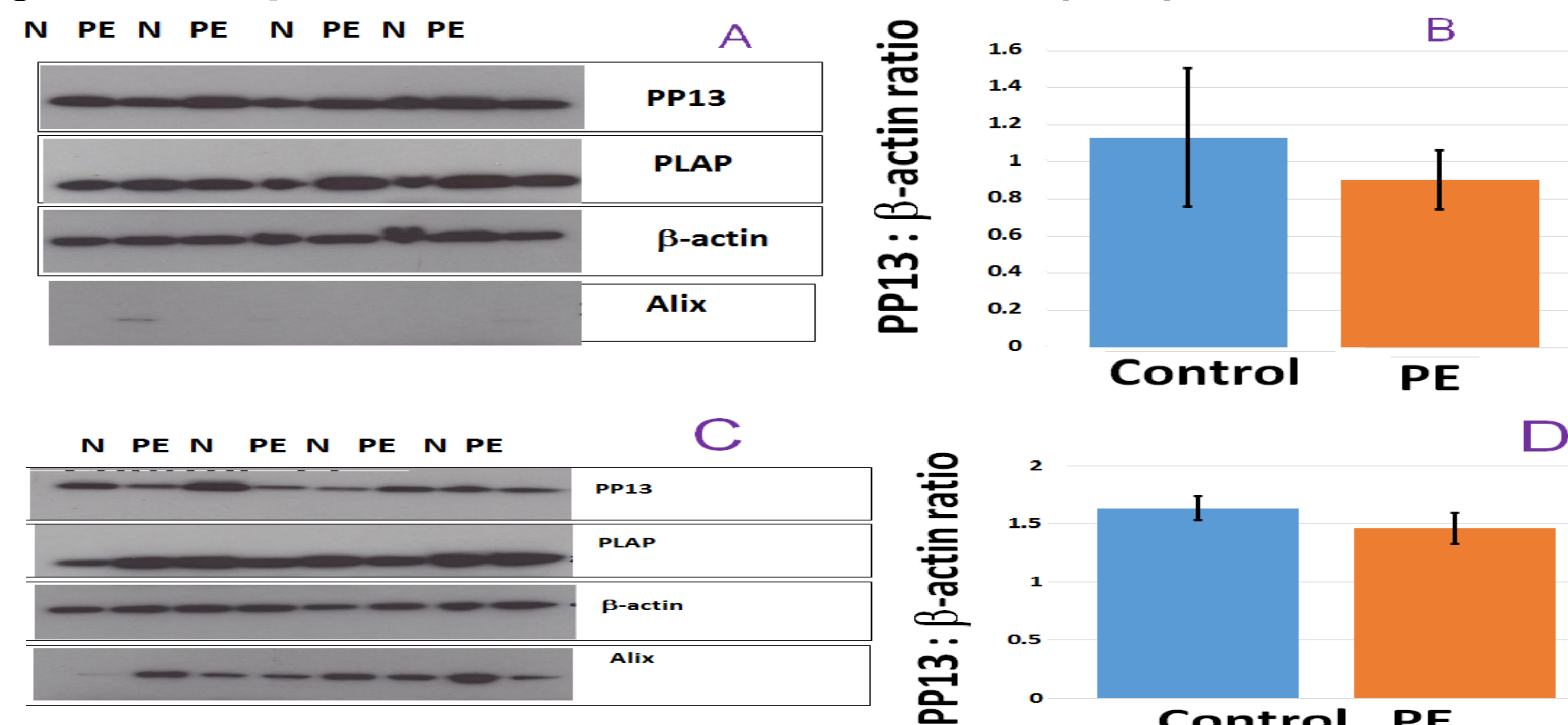
RESULTS

Figure 1: Scheme of Fractionation of Placental perfusate STB-EVs



- Two populations were isolated: 10 KP enriched for STB micro-vesicles (STB-MVs) and 150 KP enriched for STB exosomes (STB-EXs).
- Both populations are PLAP positive (NDOG2) consistent with their STB origin.
- The 150KP fraction is enriched in exosome markers Alix and CD9 but none in 10KP
- PP13 is expressed in STB-MVs and STB-EXs

Figure 2: Expression of PP13 in STB-MVs (A,B) and STB-EXs



- A and C - immunoblots of PP13 and related EV biomarkers in PE and control placental perfusate derived STB-MVs. B and D - densitometry analyses of PP13 immunoblots.
- STB-MVs (A) and STB-EX (B) derived from control and PE placenta are enriched in PLAP and β -actin .
 - ONLY STB-EX carries the exosome markers (Alix)
 - STB-MVs and STB-EXs derived from PE and control placentas contain PP13
 - Semi-quantitative analyses shows a minor non-significant reduction in PP13 level in PE compared to control placentas in both vesicular categories.

Figure 3: PP13 level in STB-EVs.

- The total PP13 load in the STBEVs (10+ 150 KP) derived from PE patients is significantly lower compared to normal placentas (300.5 ± 166.4 vs. 637.8 ± 160.8 pg/mg protein).
- The external oriented PP13 load on the STBEVs (10 + 150 KP) derived from PE patients is significantly lower compared to normal placentas (189.4 ± 136.3 vs. 308.2 ± 131.1 pg/mg protein)

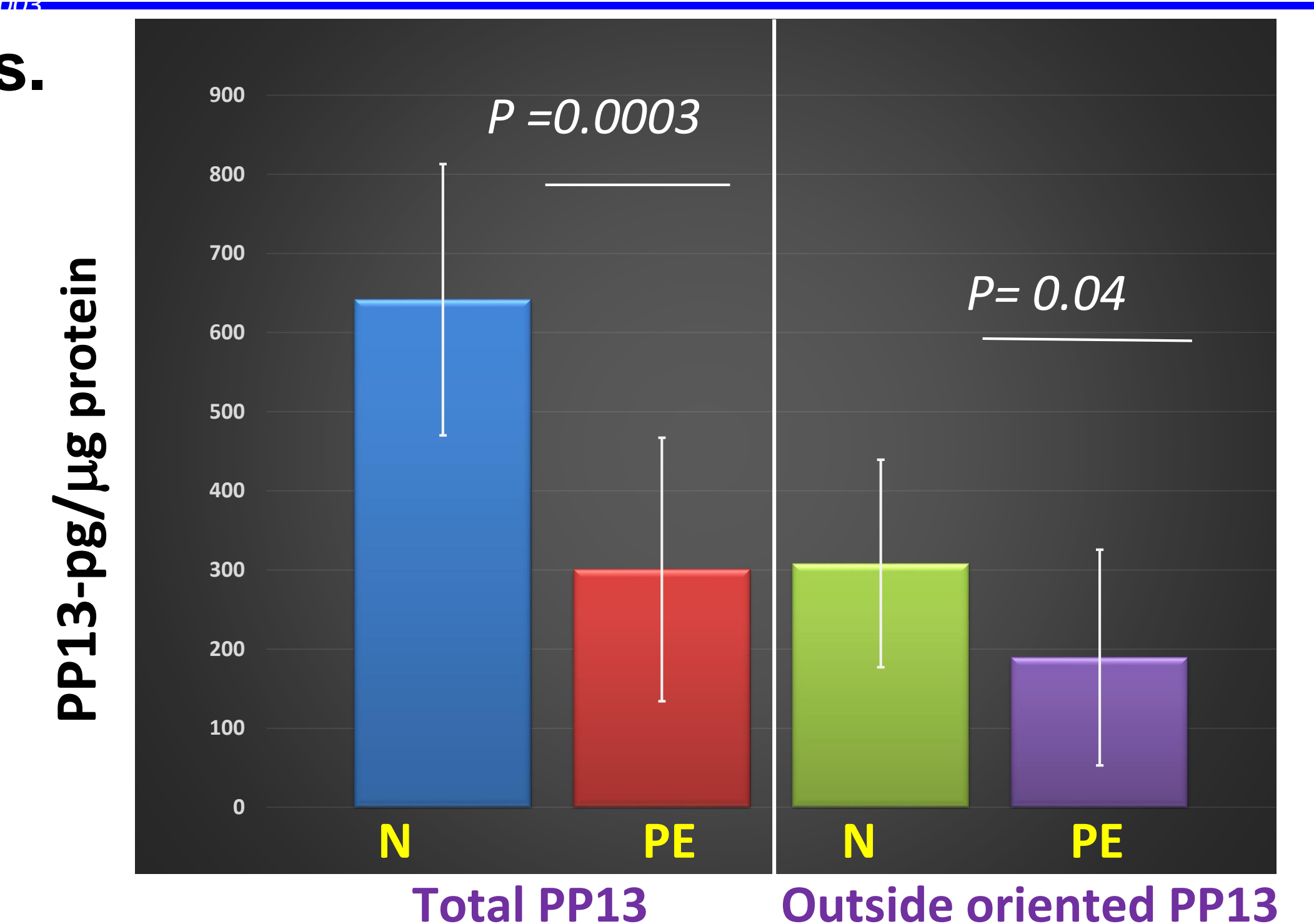
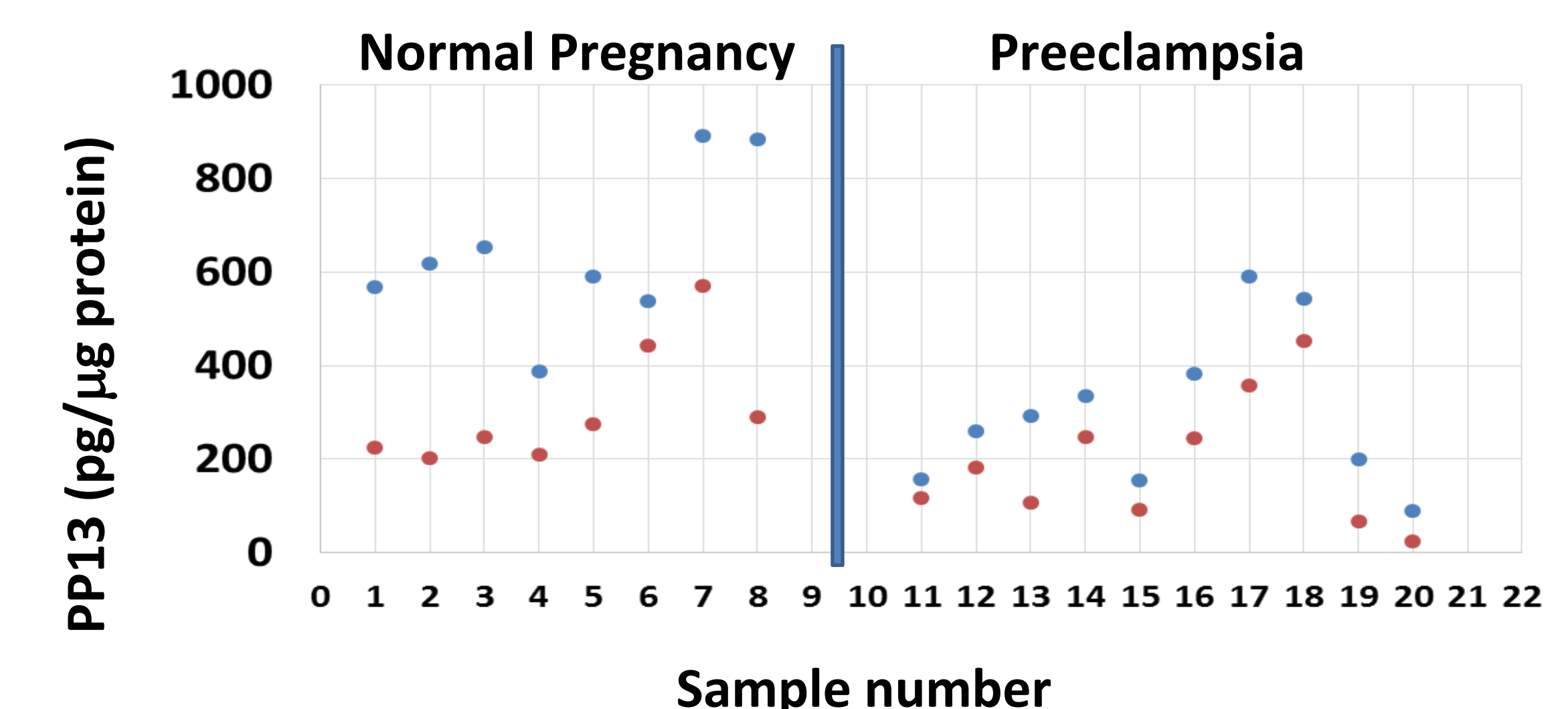


Figure 4: Localization of PP13 in the placental perfusate STB-EVs

Distribution of the total PP13 detected in solubilized STBEVs (Blue) and the external oriented PP13 displayed on the surface of STBEVs (Red). The external oriented PP13 was 49 ± 17 and 58 ± 19 % in STB-EVs derived from normal and PE placentas respectively



Conclusions

- ❑ PP13 is part of the protein cargo of STB-MVs and STB-EXs
- ❑ PP13 is localized both inside and at the outer surface of STB-EVs
- ❑ PP13 levels are lower in STB-EVs originated from PE compared to control placentae.

1- Dragovic RA, et al . *Methods*. 2015 Apr 3. pii: S1046-2023(15)00145-0. doi: 10.1016/j.ymeth.2015.03.028.

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