https://www.scientificarchives.com/journal/journal-of-cellular-immunology

Journal of Cellular Immunology Commentary

Syncytiotrophoblast Extracellular Microvesicles in Preeclampsia

Chirag Ram[1], Robert W. Hu[1], Laxminarayana Korutla[1], Andreas Habertheuer[2], Prashanth Vallabhajosyula[1*]

[1]Division of Cardiac Surgery, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut, USA

[2]Division of Cardiac Surgery, Brigham and Women's Hospital, Harvard Medical School, USA

*Correspondence should be addressed to Prashanth Vallabhajosyula; Prashanth.vallabhajosyula@yale.edu

Received date: October 07, 2020, **Accepted date:** February 19, 2021

Abstract

Preeclampsia, a placental disease, is typically characterized by hypertension and proteinuria in pregnant mothers. There is a need for improved noninvasive detection and diagnosis of this condition. Extracellular microvesicles (EVs), including exosomes, are tissue specific nanoparticles released by many tissue types including the placenta into peripheral circulation. Tissue specific EVs have potential to serve as biomarkers, and their cargoes are dynamic and may reflect functional activity of their tissue counterparts. Several groups have reported an increase in whole plasma EVs in healthy pregnant women in comparison to healthy nonpregnant women, leading to the question of, how does the preeclampsia EV profile differ? In our analysis of this condition, there was no difference in total EV quantity between samples from healthy pregnant females versus those with preeclampsia. However, when we assessed for syncytiotrophoblast EVs (STEVs) using syncytin-1 as the expressed EV surface marker for placental tissue specificity, preeclampsia subjects had significantly lower circulating STEV signal, suggesting that STEV profiles have potential to serve as a noninvasive diagnostic of preeclampsia. STEVs may also play a role in the underlying preeclampsia mechanism, where STEV protein and microRNA cargoes mediate intercellular communication in maternal tissue resulting in the onset of the disease. Coordinated, detailed investigations of STEV cargoes during early pregnancy will need to be performed to understand their potential functional roles. Overall, STEV cargo profiles provide a promising insight into the diagnosis of placental injury, particularly preeclampsia, with an opportunity to noninvasively understand their functional implications.

Keywords: Preeclampsia, Extracellular vesicles, Exosomes, Biomarkers, Syncytin-1

Introduction

This short communication is a commentary on Levine and Habertheuer's paper titled, "Syncytiotrophoblast extracellular microvesicle profiles in maternal circulation for noninvasive diagnosis of preeclampsia" published in: Scientific Reports – Nature [1].

Preeclampsia is a major cause of mortality and morbidity in expecting mothers and is one of the most common causes of pregnancy complications and premature birth [2]. Along with eclampsia, it is directly associated with 10-15% of maternal deaths [2]. Currently, it is diagnosed during the second or third trimester after clinical symptoms are displayed, but studies have attempted to understand the pathophysiologic mechanism underlying preeclampsia, noting its beginnings in the first trimester. Although there is no defined cause nor is there a cure for the disease, it is widely agreed that preeclampsia stems from the placenta and is related to a decrease in uteroplacental blood flow and hypoxia [3].

Attempts at predicting and diagnosing preeclampsia has so far been unsuccessful, with most studies investigating circulating free proteins or microRNAs. There is a need for improved noninvasive diagnosis and monitoring for preeclampsia outside the current clinical parameters. Such a biomarker would allow for preventative treatments to keep both the mother and fetus safe. It is well known that placental syncytiotrophoblasts at the maternal-fetal

interface release nanoparticles, including extracellular microvesicles (EVs), including exosomes, into the maternal blood during pregnancy [1]. Maternal EVs may provide a window for development of noninvasive biomarker platform in aiding in diagnosis and earlier surveillance of preeclampsia [4-16].

Although the exact definition of the size of microvesicles remains variable, it is well established that exosomes are nanovesicles derived from the multivesicular body ranging in size from 30 to 150 nm. They are released by most cell types and are believed to participate in cell-to-cell communication [17,18]. The first discovery of exosomes was made in the mid-1980s, where membrane-associated elements were selectively released in multivesicular body-derived circulating vesicles in maturing mammalian reticulocytes [19-21]. The first report of exosomes carrying RNAs was made in 2007, and it is now known that exosomes represent stable and tissue-specific proteomic and RNA signature profiles, including proteins, lipids, mRNAs, and microRNAs, that can reflect the conditional state of their tissue of origin [22]. Exosomes are vesicles released by many tissue types and can be found in most bodily fluids including blood, urine, bronchoalveolar secretions, saliva, and amniotic fluid [22-27]. Although exosomes were initially thought to serve as cell debris to dispose of unwanted components, increasingly investigations have demonstrated its influence on physiological and pathological processes as well as its association with diseases and treatments, implying their potential as a diagnostic tool [17].

Previously, our group investigated tissue specific EVs as a noninvasive diagnostic for early rejection in models of islet, heart, and lung transplantation [28-31]. We also recently developed methodologies for enrichment of a subpopulation of pancreatic islet β exosomes, where we can reliably track an insulin specific signal in this exosome subset [data not published]. Here, we hypothesized that syncytiotrophoblast EVs (STEVs) can serve as a biomarker for predicting the onset of placental injuries such as preeclampsia. Other groups have investigated EVs and STEVs in this context, and they have shown that healthy maternal plasma has an increase in total EVs compared to plasma from nonpregnant female. Reports have also shown that expression levels of placenta specific EVs, those expressing placental proteins syncytin-1, syncytin-2, and/or PLAP, are altered in pregnant patients experiencing placental disorders in comparison to healthy pregnancies (Table 1) [32-40].

STEVs are specifically released by the fetal-derived syncytiotrophoblast layer at the maternal-fetal interface. Therefore, profiling their quantitative and intraexosomal

Reference number	Author	Year Published	Exosome Analysis Type	Methodology	Protein Marker Used	Number of subjects studied
1	Levine, et al.	2020	Tissue specific	Western Blot and Nanoparticle detector	Syncytin-1	44
4	Bersinger, et al.	2003	Tissue specific	ELISA	PAPP-A	38
34	Pillay, et al.	2016	Tissue specific	Nanoparticle detector	PLAP	60
35	Salomon, et al.	2017	Tissue specific	Nanoparticle detector and RNA analysis	PLAP	47
37	Tan, et al.	2014	Whole plasma	ELISA, Antibody array, and Mass spectrometry	CD9, VEGFR1, BNP, ANP, PLGF	22
39	Vargas, et al.	2011	Tissue specific	Cell Fusion Assay, Real-Time RT-PCR, and Western Blot	Syncytin-1 Syncytin-2	24
40	Vargas, et al.	2014	Tissue specific	Western Blot, RT-PCR, ELISA, and Flow Cytometry	Syncytin-1 Syncytin-2	22
48	Dragovic, et al.	2015	Tissue specific	Nanoparticle detector, Western Blot, and Flow Cytometry	PLAP	8
51	Dragovic, et al.	2013	Tissue specific	Flow Cytometry, Nanoparticle detector	PLAP	10

Table 1: Analyses of preeclamspia EVs in human samples.

cargoes may reflect conditional stress imposed on the placenta, and possibly on the fetus. In this context, several placental specific proteins have been studied, especially placental alkaline phosphatase (PLAP), syncytin-1, and syncytin-2 [1, 41-47]. These STEVs are released directly into the maternal circulation, where they may influence the surrounding tissue or may have systemic effects. These protein markers have provided some interesting insights into how STEV profiles may be altered under condition of preeclampsia compared to healthy pregnancy; but at this point, their role as a noninvasive diagnostic is promising and yet to be carefully investigated.

In line with our findings in investigating the role of tissue specific exosomes in the transplantation field, we sought to understand whether STEV signal has diagnostic potential in preeclampsia. Given the known markers PLAP, plac-1, syncytin-1, and syncytin-2 reported in the literature, we first performed *in vitro* studies in BeWo human choriocarcinoma-derived cell line to check for marker expression in their EVs. It was also critical that the commercially available antibodies for these proteins that are designed for cellular protein content showed reliable and reproducible results with EV proteins. By Western blot analysis, we noted the presence of syncytin-1 and PLAP, but not plac-1, as EV surface markers. These results corroborated with nanoparticle tracking analysis, where high levels of syncytin-1 and PLAP were seen on BeWo EVs. Given these promising in vitro data, we performed analysis in maternal plasma samples to assess for STEV differential expression.

Whole plasma EVs were isolated from maternal plasma using size-exclusion column chromatography and ultracentrifugation, with samples from healthy mothers serving as a positive control. Samples were evaluated by Western blot and nanoparticle tracking analysis, along with RT-PCR analysis of EV mRNA. This study was performed with the samples blinded to the staff performing the EV experiments. First, there was no difference between healthy pregnant and preeclampsia samples when assessing for size distribution and EV quantity for whole plasma. This was consistent with our findings in the transplantation models of rejection, where whole plasma EVs were unchanged [28-30]. Western Blot analysis probing for syncytin-1 displayed a markedly higher expression in healthy pregnant EV samples compared to healthy nonpregnant EV samples; however, there was marked decrease in syncytin-1 expression for preeclampsia samples when compared to healthy pregnant samples. This observation was quantified by nanoparticle tracking analysis using anti-syncytin-1 antibody conjugated quantum dots, which strongly validated the above results. PLAP was also detected in both healthy maternal EVs and preeclampsia EVs. However, a small limitation of this study was that PLAP also reacted with non-pregnant female EVs, insinuating that there is some cross-reactivity present [1]. These findings highlighted the importance of enriching/ detecting tissue specific EVs to improve diagnostic accuracy; in this case syncytiotrophoblast EVs, as quantification of whole plasma EVs showed no quantitative, statistically significant difference between healthy pregnant and preeclampsia samples. Collectively, this demonstrated that syncytin-1 expressing EV subpopulation is significantly upregulated during healthy pregnancy. The maternal syncytin-1 EV signal is significantly decreased with preeclampsia, implicating that a tissue specific exosome platform may have diagnostic potential to reflect the placental injury associated with this condition. The finding that preeclampsia results in decreased STEV signal is consistent with our overall findings in other models where disease / injury leads to decreased detection of circulating tissue specific EVs. Similar results were noted in the transplantation setting, where immunologic rejection led to decreased donor tissue EV output.

Studying EVs, particularly STEVs in the maternal circulation, provides a novel perspective on preeclampsia, potentially providing a real time, dynamic analysis of the condition. Therefore, a detailed investigation of syncytin-1 EV expression from early pregnancy time points would provide insights into the biomarker potential of this platform. As preeclampsia is relatively frequent and occurs in 4-5% of pregnant females, a prospective study would be feasible with relative ease. In previous studies, we used tissue specific surface EV markers and successfully purified EV subsets using antibody conjugated bead technology and performed small RNA sequencing of exosomal RNA cargoes [28, 47]. These studies showed differential expression of micro RNAs in tissue specific EVs and provided functional insights into how EV micro RNA cargoes may have mechanistic implications. In this context, syncytin-1 may be utilized as a candidate marker for enrichment of a putative placental EV subpopulation, and analysis of its microRNA cargoes may provide insights into their functional roles and the potential role of STEVs in the pathophysiology of preeclampsia.

Other groups have reported differences using circulating PLAP and syncytin-2 expression, as well as whole plasma EV profiles, and the utilization of these surface markers may also provide insights into understanding putative STEV cargo profiles (Table 1) [38-41]. Our group attempted to understand PLAP specific EVs as potential STEV subpopulation, but using the commercially available antibodies to PLAP we found cross reactivity to other forms of alkaline phosphatases in circulation. According to Tannetta, et al., there is a marked difference in PLAP expression between STEVs of healthy pregnant samples

and preeclampsia samples by in vitro experiments [41]. PLAP is the most studied placental marker, but our experience with this candidate marker of STEV has been disappointing due to cross reactivity when using commercially tested antibodies.

As seen in Dragovic, et al. along with many others, nanoparticle flow cytometry can result in improved detection of target proteins specifically on STEVs, allowing for greater tissue specificity determination and better analysis of the EV cargo profiles (Table 1) [48-50]. Moreover, flow cytometry allows for greater accuracy in quantifying whole plasma EVs and STEVs when compared to nanoparticle tracking analysis, given the ability of FACS to better differentiate between background noise and the molecules of interest. But nanoparticle flow cytometry has limitations for smaller sized nanoparticles, especially exosomes, where the sensitivity and specificity of current nanoparticle flow cytometers starts to decrease. However, we anticipate that as this technology improves, nanoparticle flow cytometry would play an important role in this field in the future. In our analysis of STEVs using syncytin-1 as a marker, nanoparticle detector fluorescence and light scatter modes enabled tissue specific exosome subset detection and quantitation. This technique requires repeated sample quality validation and due to the high sensitivity of the nanoparticle detector, protein aggregates or plasma lipoproteins in the size range of exosomes 30 nm to 150 nm can lead to erroneous quantitation. Most likely, the ideal assay for STEV quantitation would be a combination of both the nanoparticle detector tracking analysis and FACS studies [51], which may help improve the overall diagnostic accuracy of STEV quantitative read out. Future studies using these techniques in larger patient cohort and performed at multiples institutions independently would help better understand the diagnostic potential of circulating STEV signal quantitation.

Furthermore, given the unique nature of the placenta as an organ that is naturally removed from the mother after giving birth facilitates performance of ex vivo perfusion experiments to better understand STEV differential profiles using syncytin-1 and PLAP as potential markers [41]. Placenta specific EVs can been isolated directly from the organ, comparing those derived from placenta of healthy subjects versus those with preeclampsia. Additionally, this would help compare and cross validate the in vivo plasma and ex vivo data. As syncytiotrophoblasts are of fetal origin, better understanding of STEV profiles may also provide novel insights into fetal disorders such as intrauterine growth retardation.

These experimental improvements will also help in better understanding the role STEVs and their cargo profiles play in the onset of preeclampsia and determine whether they are more than just a biomarker. In a recent study by Han et al., infused EVs isolated from injured placenta into healthy pregnant mice resulted in preeclampsia symptoms [52]. The mice developed both hypertension and proteinuria, which is an indication that EVs may potentially play a causative role in the development of preeclampsia. Nonpregnant healthy mice that were also infused with these EVs developed the same symptoms, which further highlights the possibility that the STEVs derived from the injured, preeclamptic placenta play a key role in communication throughout the body [52]. These experiments are corroborated by the findings of Kohli et al., as well as a previous study done by Han, et al., where infusion of EVs using mouse models resulted in an inflammatory response [53,54]. Han's group specifically used low syncytin expression on EV surface as a marker of placental injury, opening up avenues for further study [52]. These findings and methodologies can be extended to developing experimental protocols to understand the mechanisms behind the onset of preeclampsia and the means of inflammatory response in the process, furthering Han's study [55]. The ability to enrich for a putative circulating STEV population using syncytin-1 opens the door for future studies exploring these concepts.

Conclusion

Levine and Habertheuer's paper highlights the potential diagnostic utility of STEVs and the importance of tissue specificity when using EVs as a noninvasive biomarker for detection of preeclampsia. With syncytin-1 as a surface marker for EVs derived from fetal-derived syncytiotrophoblasts, our investigation showed that increased expression is a sign of a healthy pregnancy while a decrease in expression can be an indication of preeclampsia. Although currently the development and cause of preeclampsia is somewhat a mystery, recent explorations, including our group's report, have begun to investigate the utility of STEVs and their diagnostic potential. Understanding STEV cargoes may provide insights into the mechanisms underlying preeclampsia onset and progression. With the advancement of technology and research techniques, investigations on STEVs can improve to provide a deeper understanding of preeclampsia and placental pathologies as a whole.

Funding

PV is supported by K08HL132099 grant from National Heart, Lung, and Blood Institute, NIH.

Author Contributions

CR – original draft preparation, review and editing; RWH – review and editing; LK – review and editing; AH – review and editing; PV – study concept and design, review and editing.

References

1. Levine L, Habertheuer A, Ram C, Korutla L, Schwartz N, Hu RW, et al. Syncytiotrophoblast extracellular microvesicle profiles in maternal circulation for noninvasive diagnosis of preeclampsia. Sci Rep. 2020;10(1):6398.

2. Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi J. Pre-eclampsia: pathophysiology, diagnosis, and management. Vasc Health Risk Manag. 2011;467.

3. LaMarca BD, Gilbert J, Granger JP. Recent progress toward the understanding of the pathophysiology of hypertension during preeclampsia. Hypertension. 2008;51(4):982–8.

4. Bersinger NA, Smárason AK, Muttukrishna S, Groome NP, Redman CW. Women with preeclampsia have increased serum levels of pregnancy-associated plasma protein A (PAPP-A), inhibin A, activin A and soluble E-selectin. Hypertens Pregnancy. 2003;22(1):45–55.

5. Kalousová M, Muravská A, Zima T. Pregnancy-associated plasma protein A (PAPP-A) and preeclampsia. Adv Clin Chem. 2014;63:169–209.

6. Poon LCY, Maiz N, Valencia C, Plasencia W, Nicolaides KH. First-trimester maternal serum pregnancy-associated plasma protein-A and pre-eclampsia. Ultrasound Obstet Gynecol. 2009;33(1):23–33.

7. Sung KU, Roh JA, Eoh KJ, Kim EH. Maternal serum placental growth factor and pregnancy-associated plasma protein A measured in the first trimester as parameters of subsequent pre-eclampsia and small-for-gestational-age infants: A prospective observational study. Obstet Gynecol Sci. 2017;60(2):154–62.

8. Cozzi V, Garlanda C, Nebuloni M, Maina V, Martinelli A, Calabrese S, et al. PTX3 as a potential endothelial dysfunction biomarker for severity of preeclampsia and IUGR. Placenta. 2012;33(12):1039–44.

9. Carty DM, Delles C, Dominiczak AF. Novel biomarkers for predicting preeclampsia. Trends Cardiovasc Med. 2008;18(5):186–94.

10. Ree PH, Hahn WB, Chang SW, Jung SH, Kang JH, Cha DH, et al. Early detection of preeclampsia using inhibin a and other second-trimester serum markers. Fetal Diagn Ther. 2011;29(4):280–6.

11. Teixeira PG, Cabral ACV, Andrade SP, Reis ZSN, da Cruz LPB, Pereira JB, et al. Placental growth factor (PlGF) is a surrogate marker in preeclamptic hypertension. Hypertens Pregnancy. 2008;27(1):65–73.

12. Keshavarzi F, Mohammadpour-Gharehbagh A, Shahrakipour M, Teimoori B, Yazdi A, Yaghmaei M, et al. The placental vascular endothelial growth factor polymorphisms and preeclampsia/preeclampsia severity. Clin Exp Hypertens. 2017;39(7):606–11.

13. Taché V, LaCoursiere DY, Saleemuddin A, Parast MM. Placental expression of vascular endothelial growth factor receptor-1/soluble vascular endothelial growth factor receptor-1 correlates with severity of clinical preeclampsia and villous hypermaturity. Hum Pathol. 2011;42(9):1283–8.

14. Fu G, Ye G, Nadeem L, Ji L, Manchanda T, Wang Y, et al. MicroRNA-376c impairs transforming growth factor-β and nodal signaling to promote trophoblast cell proliferation and invasion. Hypertension. 2013;61(4):864–72.

15. Jiang L, Long A, Tan L, Hong M, Wu J, Cai L, et al. Elevated microRNA-520g in pre-eclampsia inhibits migration and invasion of trophoblasts. Placenta. 2017;51:70–5.

16. Andjus P, Kosanović M, Milićević K, Gautam M, Vainio SJ, Jagečić D, et al. Extracellular vesicles as innovative tool for diagnosis, regeneration and protection against neurological damage. Int J Mol Sci [Internet]. 2020;21(18). Available from: http://dx.doi.org/10.3390/ijms21186859

17. Lin J, Li J, Huang B, Liu J, Chen X, Chen X-M, et al. Exosomes: novel biomarkers for clinical diagnosis. ScientificWorldJournal. 2015;2015:657086.

18. Ma C, Jiang F, Ma Y, Wang J, Li H, Zhang J. Isolation and detection technologies of extracellular vesicles and application on cancer diagnostic. Dose Response. 2019;17(4):1559325819891004.

19. Johnstone RM. Maturation of reticulocytes: formation of exosomes as a mechanism for shedding membrane proteins. Biochem Cell Biol. 1992;70(3–4):179–90.

20. Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: selective externalization of the receptor. Cell. 1983;33(3):967–78.

21. Pan BT, Teng K, Wu C, Adam M, Johnstone RM. Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. J Cell Biol. 1985;101(3):942–8.

22. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9(6):654–9.

23. Johann DJ Jr, Blonder J. Biomarker discovery: tissues versus fluids versus both. Expert Rev Mol Diagn. 2007;7(5):473–5.

24. Julich H, Willms A, Lukacs-Kornek V, Kornek M. Extracellular vesicle profiling and their use as potential disease specific biomarker. Front Immunol. 2014;5:413.

25. Lakkaraju A, Rodriguez-Boulan E. Itinerant exosomes: emerging roles in cell and tissue polarity. Trends Cell Biol. 2008;18(5):199–209.

26. Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. J Proteomics. 2010;73(10):1907–20.

27. Lötvall J, Hill AF, Hochberg F, Buzás EI, Di Vizio D, Gardiner C, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. J Extracell Vesicles. 2014;3(1):26913.

28. Vallabhajosyula P, Korutla L, Habertheuer A, Yu M, Rostami S, Yuan C-X, et al. Tissue-specific exosome biomarkers for noninvasively monitoring immunologic rejection of transplanted tissue. J Clin Invest. 2017;127(4):1375–91.

29. Korutla L, Rickels MR, Hu RW, Freas A, Reddy S, Habertheuer A, et al. Noninvasive diagnosis of recurrent autoimmune type 1 diabetes after islet cell transplantation. Am J Transplant. 2019;19(6):1852–8.

30. Habertheuer A, Korutla L, Rostami S, Reddy S, Lal P, Naji A, et al. Donor tissue-specific exosome profiling enables noninvasive monitoring of acute rejection in mouse allogeneic heart transplantation. J Thorac Cardiovasc Surg. 2018;155(6):2479–89.

31. Habertheuer A, Japp A, Ram C, Chatterjee S, Rostami S, et al. Pleural exosome mediated donor antigen trafficking into mediastinal lymph nodes represents a novel pathway of recipient T cell activation in lung transplantation. In: ATC2020. American Transplant Congress; 2020 May 30 – Jun 1; Philadelphia, PA.

32. Mitchell MD, Peiris HN, Kobayashi M, Koh YQ, Duncombe G, Illanes SE, et al. Placental exosomes in normal and complicated pregnancy. Am J Obstet Gynecol. 2015;213(4):S173–81.

33. Cuffe JSM, Holland O, Salomon C, Rice GE, Perkins AV. Review: Placental derived biomarkers of pregnancy disorders. Placenta. 2017;54:104–10.

34. Pillay P, Maharaj N, Moodley J, Mackraj I. Placental exosomes and pre-eclampsia: Maternal circulating levels in normal pregnancies and, early and late onset pre-eclamptic pregnancies. Placenta. 2016;46:18–25.

35. Salomon C, Guanzon D, Scholz-Romero K, Longo S, Correa P, Illanes SE, et al. Placental exosomes as early biomarker of preeclampsia: Potential role of exosomal MicroRNAs across gestation. J Clin Endocrinol Metab. 2017;102(9):3182–94.

36. Salomon C, Rice GE. Role of exosomes in placental homeostasis and pregnancy disorders. Prog Mol Biol Transl Sci. 2017;145:163–79.

37. Tan KH, Tan SS, Sze SK, Lee WKR, Ng MJ, Lim SK. Plasma biomarker discovery in preeclampsia using a novel differential isolation technology for circulating extracellular vesicles. Am J Obstet Gynecol. 2014;211(4):380.e1-13.

38. Vargas A, Moreau J, Landry S, LeBellego F, Toufaily C, Rassart E, et al. Syncytin-2 plays an important role in the fusion of human trophoblast cells. J Mol Biol. 2009;392(2):301–18.

39. Vargas A, Toufaily C, LeBellego F, Rassart É, Lafond J, Barbeau B. Reduced expression of both syncytin 1 and syncytin 2 correlates with severity of preeclampsia. Reprod Sci. 2011;18(11):1085–91.

40. Vargas A, Zhou S, Éthier-Chiasson M, Flipo D, Lafond J, Gilbert C, et al. Syncytin proteins incorporated in placenta exosomes are important for cell uptake and show variation in abundance in serum exosomes from patients with preeclampsia. FASEB J. 2014;28(8):3703–19.

41. Tannetta D, Collett G, Vatish M, Redman C, Sargent I. Syncytiotrophoblast extracellular vesicles – Circulating biopsies reflecting placental health. Placenta. 2017;52:134–8.

42. Miranda J, Paules C, Nair S, Lai A, Palma C, Scholz-Romero K, et al. Placental exosomes profile in maternal and fetal circulation in intrauterine growth restriction - Liquid biopsies to monitoring fetal growth. Placenta. 2018;64:34–43.

43. Qiao S, Wang F, Chen H, Jiang S-W. Inducible knockout of Syncytin-A gene leads to an extensive placental vasculature deficiency, implications for preeclampsia. Clin Chim Acta. 2017;474:137–46.

44. Salomon C, Torres MJ, Kobayashi M, Scholz-Romero K, Sobrevia L, Dobierzewska A, et al. A gestational profile of placental exosomes in maternal plasma and their effects on endothelial cell migration. PLoS One. 2014;9(6):e98667.

45. Sarker S, Scholz-Romero K, Perez A, Illanes SE,

Mitchell MD, Rice GE, et al. Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. J Transl Med. 2014;12(1):204.

46. Menon R, Debnath C, Lai A, Guanzon D, Bhatnagar S, Kshetrapal P, et al. Protein profile changes in circulating placental extracellular vesicles in term and preterm births: A longitudinal study. Endocrinology [Internet]. 2020;161(4). Available from: http://dx.doi.org/10.1210/endocr/bqaa009

47. Saha P, Sharma S, Korutla L, Datla SR, Shoja-Taheri F, Mishra R, et al. Circulating exosomes derived from transplanted progenitor cells aid the functional recovery of ischemic myocardium. Sci Transl Med. 2019;11(493):eaau1168.

48. Dragovic RA, Collett GP, Hole P, Ferguson DJP, Redman CW, Sargent IL, et al. Isolation of syncytiotrophoblast microvesicles and exosomes and their characterisation by multicolour flow cytometry and fluorescence Nanoparticle Tracking Analysis. Methods. 2015;87:64–74.

49. Koritzinsky EH, Street JM, Star RA, Yuen PST. Quantification of exosomes: Quantification of exosomes. J Cell Physiol. 2017;232(7):1587–90.

50. Morgan TK. Cell- and size-specific analysis of placental extracellular vesicles in maternal plasma and pre-eclampsia. Transl Res. 2018;201:40–8.

51. Dragovic RA, Southcombe JH, Tannetta DS, Redman CWG, Sargent IL. Multicolor flow cytometry and nanoparticle tracking analysis of extracellular vesicles in the plasma of normal pregnant and pre-eclamptic women. Biol Reprod. 2013;89(6):151.

52. Han C, Wang C, Chen Y, Wang J, Xu X, Hilton T, et al. Placenta-derived extracellular vesicles induce preeclampsia in mouse models. Haematologica. 2020;105(6):1686–94.

53. Kohli S, Ranjan S, Hoffmann J, Kashif M, Daniel EA, Al-Dabet MM, et al. Maternal extracellular vesicles and platelets promote preeclampsia via inflammasome activation in trophoblasts. Blood. 2016;128(17):2153–64.

54. Han C, Han L, Huang P, Chen Y, Wang Y, Xue F. Syncytiotrophoblast-derived extracellular vesicles in pathophysiology of preeclampsia. Front Physiol. 2019;10:1236.

55. Saito S, Shiozaki A, Nakashima A, Sakai M, Sasaki Y. The role of the immune system in preeclampsia. Mol Aspects Med. 2007;28(2):192–209.