
Article:

Farrelly, R, Kennedy, MG, Spencer, R orcid.org/0000-0002-3019-0322 et al. (1 more author) (2023) Extracellular vesicles as markers and mediators of pregnancy complications: gestational diabetes, pre-eclampsia, preterm birth and fetal growth restriction. The Journal of Physiology. ISSN 0022-3751

https://doi.org/10.1113/jp282849

Title:

Extracellular vesicles as markers and mediators of pregnancy complications: gestational diabetes, pre-eclampsia, preterm birth and fetal growth restriction.

Authors:

Name	Institution	Email
Rachel Farrelly	Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, UK	um18rf@leeds.ac.uk
Margeurite Gina Kennedy		bs15mgk@leeds.ac.uk
Rebecca Spencer		r.n.spencer@leeds.ac.uk
Karen Forbes		k.a.forbes@leeds.ac.uk

Abstract:

In high income countries, approximately 10% of pregnancies are complicated by pre-eclampsia (PE), preterm birth (PTB), fetal growth restriction (FGR) and/or macrosomia resulting from gestational diabetes (GDM). Despite the burden of disease this places on pregnant people and their newborns, there are still few, if any, effective ways of preventing or treating these conditions. There are also gaps in our understanding of the underlying pathophysiologies and our ability to predict which mothers will be affected. The placenta plays a crucial role in pregnancy and alterations in placental structure and function have been implicated in all of these conditions. As extracellular vesicles (EVs) have emerged as important molecules in cell-to-cell communication in health and disease, recent research involving maternal- and placental-derived EVs (pEVs) has demonstrated their potential as predictive and diagnostic biomarkers of obstetric disorders. This review will consider how placental and maternal EVs have been investigated in pregnancies complicated by PE, PTB, FGR and GDM and aims to highlight areas where further research is required to enhance the management and eventual treatment of these pathologies.

This is an Accepted Article that has been peer-reviewed and approved for publication in The Journal of Physiology, but has yet to undergo copy-editing and proof correction. Please cite this article as an 'Accepted Article'; doi: 10.1113/jphysiol.2023.15552.

Conflicts of interest:

None

Funding

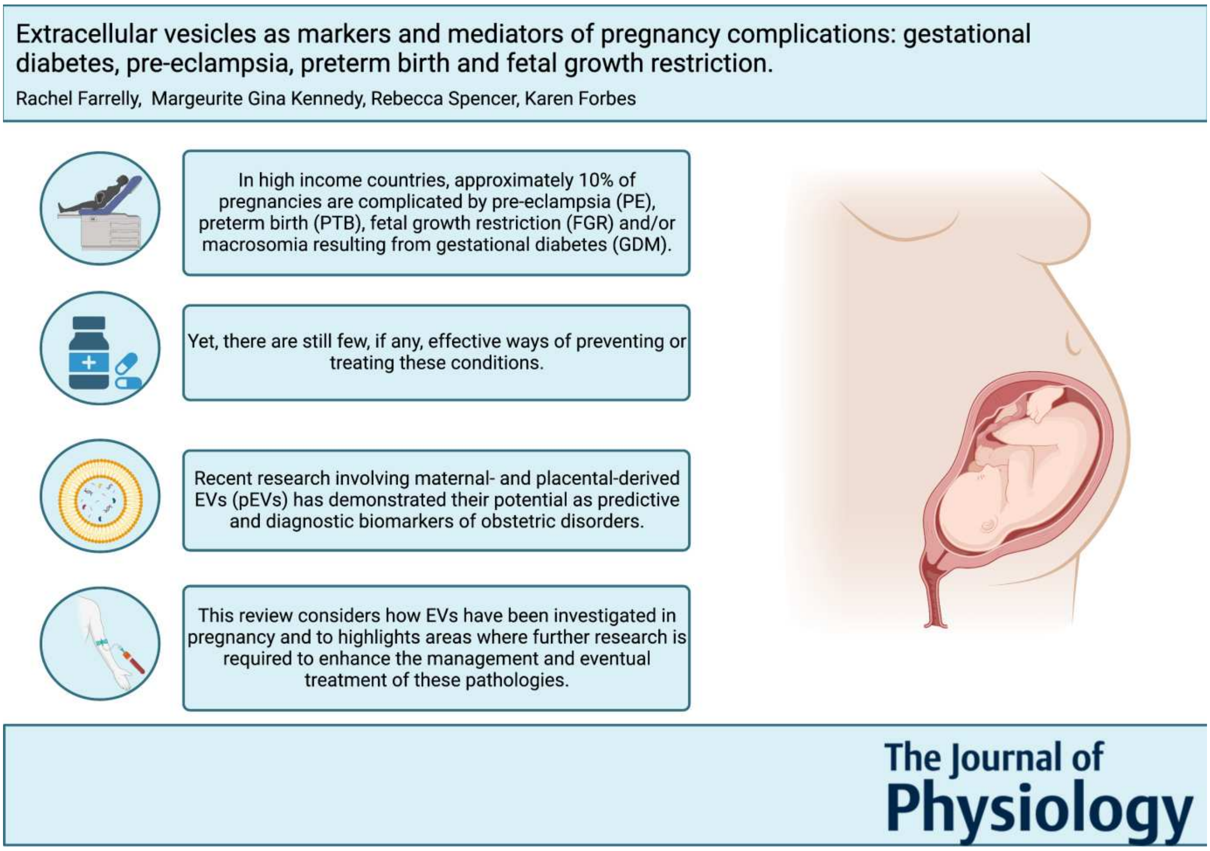
RF – None

MK – Mary and Alice Smith University of Leeds scholarship

RS – NIHR, Academy of Medical Sciences Starter Grant SGL026\1008

KF and MK – Medical Research Council (MRC) New Investigator Grant awarded to KF (REF:MR/R023166/1)

Abstract figure legend Graphical abstract summarising the salient points covered within the topical literature review.



Introduction

In high income countries, approximately 10% of pregnancies are complicated by pre-eclampsia (PE), preterm birth (PTB), fetal growth restriction (FGR) and/or large-for-gestational age (LGA) infants resulting from gestational diabetes (GDM) (Table 1). Despite the burden of disease they place on pregnant people and their newborns, there are still few, if any, effective ways of preventing or treating these conditions. Current management relies on clinical surveillance and optimising the time, place and route of delivery (Alberry & Soothill, 2007; Goldenberg *et al.*, 2008; Burton *et al.*, 2019; Quintanilla Rodriguez & Mahdy, 2022). These obstetric conditions are not only responsible for maternal, fetal and neonatal morbidity and mortality; they are also associated with long-term increased risks of cardiometabolic disease in the mothers and children (Lees *et al.*, 2013; Colella *et al.*, 2018; Graves *et al.*, 2019; Bendix *et al.*, 2020).

There is a growing body of evidence suggesting extracellular vesicles (EVs) play an important role in communication between the mother, placenta and fetus (Tong & Chamley, 2015; Chiarello *et al.*, 2018). This makes them a promising means of investigating the mechanisms underlying obstetric diseases, as well as a potential source of predictive, diagnostic and prognostic biomarkers (Familari *et al.*, 2017; Miranda *et al.*, 2018a). It may also be possible to harness their capacity for inter-organ communication for use in future therapeutics (Merino-Gonzalez *et al.*, 2016; Keshtkar *et al.*, 2018). This review aims to highlight the recent advances in the study of extracellular vesicles in pregnancy with a particular focus on gestational diabetes, pre-eclampsia, preterm birth and fetal growth restriction. It also aims to expose gaps in our current understanding and potential areas for future study in the pursuit of treatment for these pathologies.

Table 1.Brief overview of the obstetric conditions discussed in this article. The definitions and diagnostic criteria provided are widely used but not universally agreed.

Obstetric Condition	Definition	Diagnosis
Gestational diabetes	Glucose intolerance above an agreed threshold that develops during pregnancy and usually resolves after delivery	Elevated fasting plasma glucose and/or elevated one or two hour plasma glucose following a glucose tolerance test (thresholds vary) (National Institute for Health and Care Excellence, 2015 (updated 2020); Kapur *et al.*, 2020)
Pre-eclampsia	A multisystem disorder characterised by new-onset hypertension at 20+0 weeks of pregnancy or later with one or more additional features	Blood pressure ≥140/≥90 mmHg with proteinuria and/or evidence of maternal acute kidney injury, liver dysfunction, neurological features, haemolysis or thrombocytopenia, and/or fetal growth restriction (Brown *et al.*, 2018; American College of Obstetricians and Gynecologists, 2020)
Preterm birth	Birth before 37 completed weeks of gestation	Birth before 37 completed weeks of gestation
Fetal growth restriction	A failure of the fetus to reach its growth potential. (Malhotra *et al.*, 2019)	Estimated fetal weight (EFW) or abdominal circumference (AC) < 3rd centile or EFW or AC <10th centile with abnormal Doppler velocimetry and/or slowing of fetal growth (Gordijn *et al.*, 2018)

The Placenta

The placenta plays a central role in healthy pregnancy and is both a source and recipient of EVs. From 16 weeks of gestation the placenta comprises a supporting mesodermal core, containing an extensive network of fetal blood vessels (Figure 1) (Kingdom *et al.*, 2000). These form a branching, villous structure overlaid by the syncytiotrophoblast, a continuously replenished syncytium that sheds fragments into the maternal circulation. Placental angiogenesis and vasculogenesis is heavily influenced by members of the vascular endothelial growth factor (VEGF) family, including placental growth factor (PlGF) and VEGF-A, and their receptors (Umapathy *et al.*, 2019).

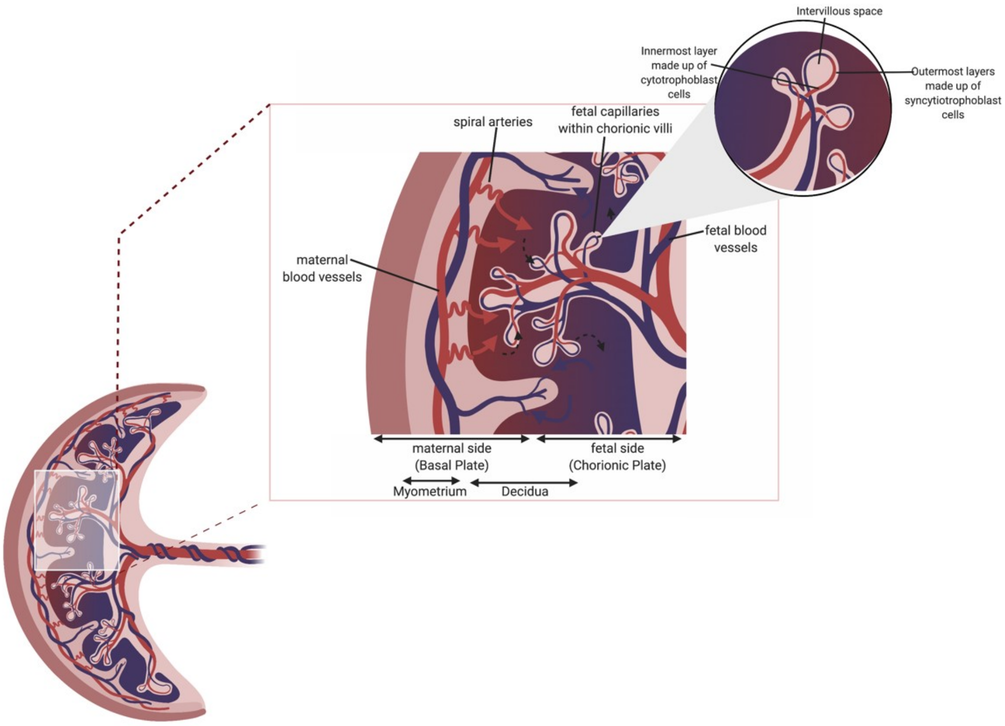


Figure 1. Anatomical cross-section of the human placenta adapted from (Jansen et al., 2020). Created in Biorender.com

Maternal blood enters the intervillous space via the spiral arteries to supply the fetus and placenta with oxygen and nutrients. During pregnancy the spiral arteries are remodelled following extravillous trophoblast invasion, under the influence of decidual immune cells, to reduce vascular resistance and increase flow velocity (Cartwright *et al.*, 2010b; Williams *et al.*, 2009). Incomplete spiral artery remodelling has been associated with early-onset fetal growth restriction and pre-eclampsia (Figure 2) (Cartwright *et al.*, 2010a) due to impaired placental function, however and the precise mechanisms responsible for this remain elusive.

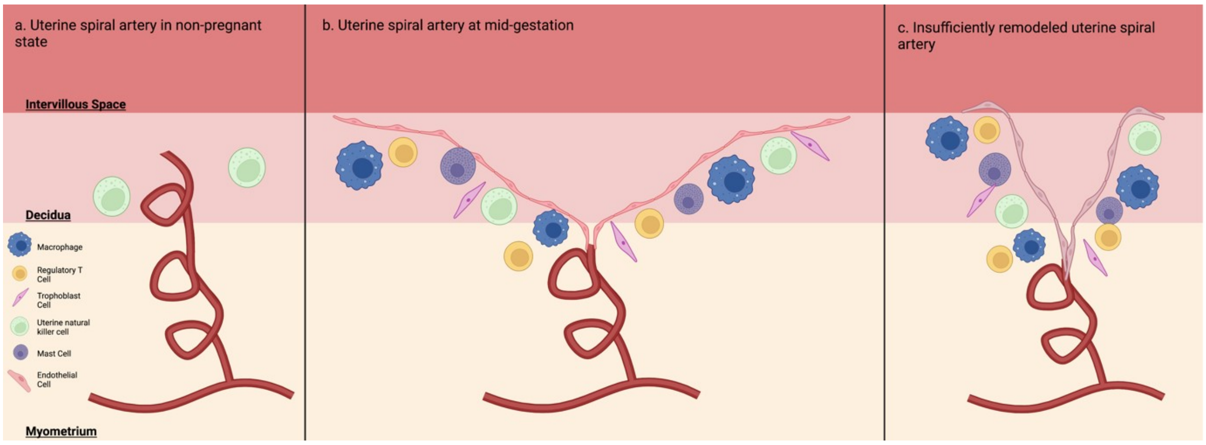


Figure 2. Schematic diagram of the spiral artery in non-pregnant state, healthy pregnancy, and pregnancies with insufficiently remodelled spiral arteries. A number of other cells in addition to trophoblast cells are thought to be involved in the vascular remodelling of the spiral artery. These include macrophages, uterine natural killer cells (uNK) and uterine mast cells. Adapted from (Schumacher et al., 2018). Created in Biorender.com

One of the major challenges in studying normal and abnormal placental function is the inaccessibility of the placenta during pregnancy. Placental sampling during pregnancy carries a risk of miscarriage or preterm birth, so placentas from uncomplicated pregnancies can only be analysed following term deliveries. Therefore, placentas from spontaneous or iatrogenic preterm deliveries cannot be compared to 'normal' placentas of the same gestation. Analysis of placental EVs offers a possible solution to this challenge.

Extracellular Vesicles

Extracellular vesicles are a non-replicating, lipid bilayer delimited particles produced by all cells for cell-to-cell communication (Yáñez-Mó *et al.*, 2015). They are transported within extracellular spaces, in biofluids such as amniotic fluid (Balbi *et al.*, 2017; Hell *et al.*, 2017) and in plasma (Arraud *et al.*, 2014). EVs are characterised by their pathway of release from cells into three subtypes of overlapping sizes: exosomes (~40-150nm), microvesicles (~100-1000nm) and apoptotic bodies (~500-2000nm; Figure. 3 (Skotland *et al.*, 2017). Each of these subtypes is distinguished by its mechanism of biogenesis, exosomes, for example, are generated from the inward budding of the endosomal membrane to form a multivesicular body (MVB) (Skotland *et al.*, 2017; McVey & Kuebler, 2018). The MVB then releases these intraluminal vesicles as exosomes upon fusion with the plasma membrane. Microvesicles, on the other hand, are derived from the outward budding of the plasma membrane and are typically released under conditions of cellular stress and activation (Skotland *et al.*, 2017; McVey & Kuebler, 2018). Lastly, apoptotic bodies are formed during apoptosis, where cellular components are fragmented and packaged into EVs (Kalra *et al.*, 2016)

.

Due to the overlapping size distributions of the subtypes, in combination with the lack of a definitive specific marker for each subtype, the field is now moving away from using these terms to describe EVs in publications (Lancaster & Febbraio, 2005; Théry *et al.*, 2018). The 'Minimal Information for Studies of Extracellular Vesicles' set by the International Society for Extracellular Vesicles (ISEV) instead recommends that EVs are described by their physical characteristics, ergo, their size with defined ranges that do not overlap (Théry *et al.*, 2018). For example, EVs <200nm in diameter are now referred to as small EVs (sEV) and EVs >200nm are large EVs (LEVs).

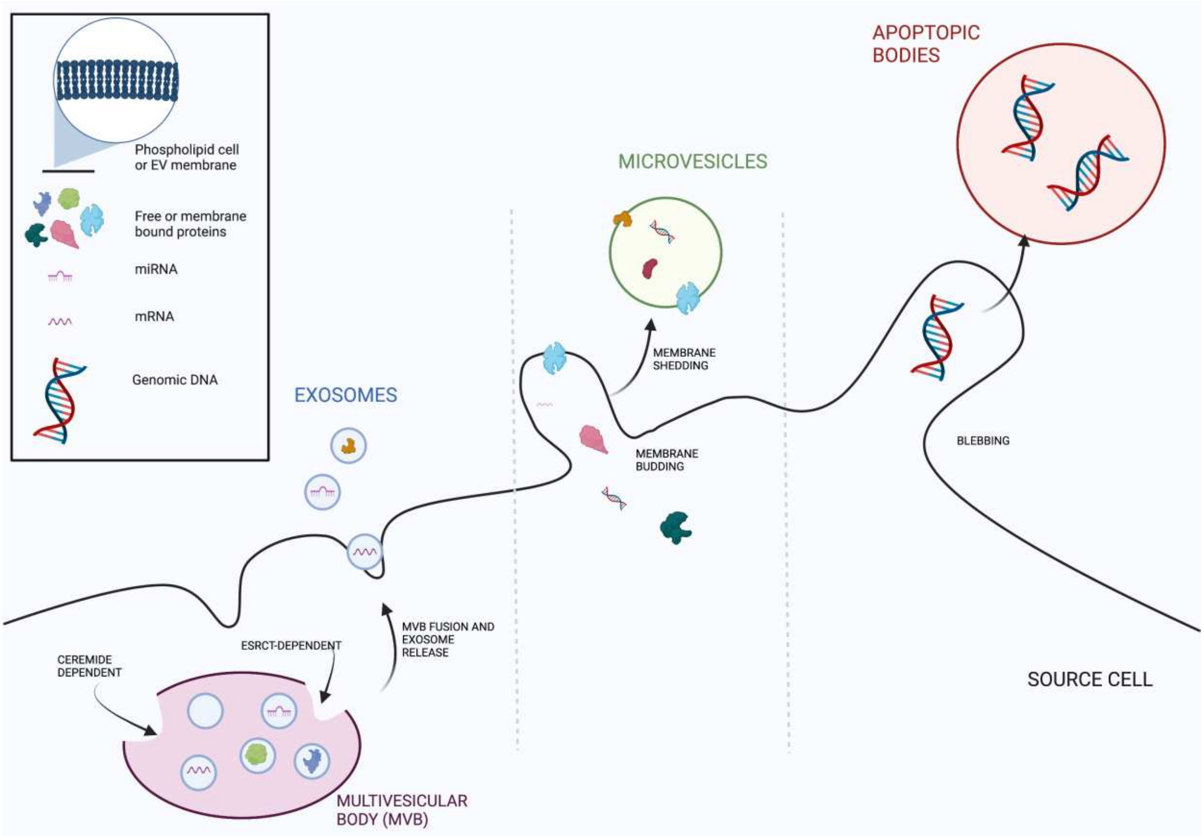


Figure 3. Biogenesis of Exosomes, Microvesicles and Apoptotic Bodies (small, medium and large EVs (El Andaloussi et al., 2013)). Exosomes range from 30-150nm in diameter and are released from the multivesicular body through the fusion with the cell membrane, they show the same membrane orientation as the source cells. Whilst this is also the case with microvesicles (100nm-1μm in diameter), they are formed from a heterogenous process involving the budding of a cell membrane around the intended contents and the shedding of this membrane. Apoptotic bodies range from 50nm-5000nm in diameter, (Doyle & Wang, 2019) They are formed by the separation of source cell plasma from the cytoskeleton as a reaction to increased hydrostatic pressure after cell contraction (Wickman et al., 2012). Created with Biorender.com

Extracellular Vesicle Cargo

All EVs transport a range of cellular cargo, including phospholipids, miRNA, mRNA, DNA, and transmembrane as well as cytosolic proteins (Kalra *et al.*, 2016; Sáez *et al.*, 2018). In 2007, a role for EVs as mediators of cell-cell communication was first described when Valadi et al. demonstrated that EVs can transfer mRNA from one cell to another, leading to protein transcription and hence a potentially functional effect (Lotvall & Valadi, 2007; Valadi *et al.*, 2007). It is now well established that in addition to mRNA, EVs transport their other cargo, including protein, lipid and non- coding RNAs between cells from different areas of the body and as such, they are key regulators of cellular communication (Boon & Vickers, 2013; McVey & Kuebler, 2018).

In addition to their key roles in cell-cell communication, the stablility of EVs and their ability to protect their cargo, specifically miRNAs, from degradation (Ge *et al.*, 2014) has sparked research interest into the use of EVs as diagnostic biomarkers (Simeone *et al.*, 2020; Zhao & Yang, 2021), as indicators of disease progression (Lee *et al.*, 2021; Simionescu *et al.*, 2021) and the preliminary exploration at targeted therapies (D'Arrigo *et al.*, 2019; Pezzana *et al.*, 2021). The benefit of EVs over non-vesicular methods of transportation is that the phospholipid bilayer protects cargo and could allow for tissue-specific delivery (Hoshino *et al.*, 2015; Jiao *et al.*, 2017; Manier *et al.*, 2017).

Whilst the role of EVs as biomarkers and functional mediators of cell-cell communication in different physiological and pathological conditions, including in pregnancy, has only emerged in recent years, the production of EVs from the placenta has been long established. During normal pregnancy the syncytiotrophobast releases EVs of different sizes, including macro-vesicles (such as syncytial nuclear aggregates (SNAs), micro-vesicles (MVs) and exosomes, into maternal circulation (Out *et al.*, 1991; Heazell *et al.*, 2007; Warrander *et al.*, 2012). Much of the original work that examined EVs in pregnancy primarily focussed on large EVs such as MVs and SNAs; establishing that levels increase across gestation and that they have key roles in the pathogenesis of pre-eclampsia by influencing the maternal immune response (Holder *et al.*, 2012; Holder *et al.*, 2016). In more recent years, the focus has shifted to study small EVs in obstetric complications

The role of these large EVs in pregnancy is well reviewed (Redman & Sargent, 2008; Redman *et al.*, 2012) and the study of small EVs continues to be the most commonly studied EV subtype across multiple conditions including cancer (Azmi et al., 2013; Xu et al., 2018 ; Lee et.al., 2021 ; Hoshino et al., 2015), heart disease (Bei et al., 2017; Saheera et al., 2021; Akhmerov et al., 2022) and obstetric complications. Therefore, whilst some of the discussed work may include large EVs, due overlap in size categorisation between small EVs and MVs, the main focus will be on sEVs.

Extracellular vesicles in healthy pregnancy

Endometrial, embryonic and placental EVs all contribute to early pregnancy development, influencing implantation, immunomodulation and spiral artery remodelling (Andronico *et al.*, 2019; Zhang *et al.*, 2020; Chen *et al.*, 2022; Morelli & Sadovsky, 2022). Endometrial epithelial EVs are present in the uterine fluid of humans and other animals (Li *et al.*, 2021; Mishra *et al.*, 2021). EVs from cultured endometrial epithelium are able to enter trophectoderm cells (Evans *et al.*, 2019) and have been shown to increase blastocyst adhesion and invasion and promote embryo development (Mishra *et al.*, 2021). Conversely, EVs from cultured embryos can enter endometrial epithelial cells (Giacomini *et al.*, 2017) and trophoblast EVs are readily taken up by monocytes, increasing migration and altering cytokine production (Atay *et al.*, 2011). Placental EVs may also be responsible for presenting paternal minor histocompatility antigens to maternal T and B cells in such a way that they produce immunotolerance (Morelli & Sadovsky, 2022).

As discussed, spiral artery remodelling is important for meeting the metabolic demands of the fetus and placenta. Salomon et al. have demonstrated the capacity for extravillous trophoblast-derived EVs to increase vascular smooth muscle cell migration (Salomon *et al.*, 2014b), while Jia and colleagues found that both maternal and umbilical cord derived porcine EVs could significantly enhance the migration and proliferation of human umbilical vein endothelial cells (Jia *et al.*, 2018). Placental EVs have also been found to contain and release vascular endothelial growth factor A (VEGF-A), a member of the VEGF family and one of the major contributors to angiogenesis and vasculogenesis (Figure 2) (Patton *et al.*, 2015; Condrat *et al.*, 2021).

Bidirectional Extracellular Vesicle Trafficking via the Placenta

The placenta releases EVs into the maternal and fetal circulations, takes up maternal EVs and allows transit of maternal and exogenous EVs to the fetus (Chiarello *et al.*, 2018; Buca *et al.*, 2020). The capacity for pEVs to enter maternal cells has been demonstrated by Tong et al. who found uptake of fluorescently-labelled EVs from cultured placenta localised to maternal lungs, liver and kidneys after venous administration in pregnant mice (Tong *et al.*, 2017). The concentration of placental EVs (pEVs) in maternal plasma increases over the course of pregnancy, as indicated by exosomal placental alkaline phosphatase (PLAP) concentration (Salomon *et al.*, 2014a), with 12-25% of all maternal circulating EVs being of placental origin (Elfeky *et al.*, 2017). Bidirectional trafficking of placental specific chromosome 14 and chromosome 19 cluster miRNAs into maternal and fetal compartments has been demonstrated both in vivo in mice, as well as in matched patient placental biopsies, maternal and fetal plasma (Chang *et al.*, 2017 2018; Paquette *et al.*, 2018).

Holder et al.'s visualisation of the internalisation of fluorescent-labelled maternal macrophage-derived EVs by the placenta shows that this EV- mediated communication between maternal tissues and the placenta is bidirectional. They also found maternal macrophage EVs that modulate placental cytokine production were actively transported into the placenta by clathrin- mediated endocytosis (Holder, 2016). This bidirectional communication is supported by the ability of maternal adipose tissue EVs to influence glucose metabolism in the placenta, by upregulating genes involved in glycolysis and gluconeogenesis (Jayabalan, 2019) EVs cargo from dietary origin have also been shown to enter into and influence events in the placenta, highlighting the potential for dietary-derived EVs/cargo to to reach even distal organs (Timms *et al.*, 2022).

Extracellular vesicles in obstetric diseases

Given the increasing evidence for EV function in healthy pregnancy, it is unsurprising that interest in the roles of EVs in obstetric diseases has also grown. This includes both their possible actions as mediators of pregnancy complications and their potential as a source of protein and RNA biomarkers to predict and diagnose disease.

Extracellular vesicles in Gestational Diabetes

Gestational diabetes mellitus (GDM) affects 6% of pregnancies worldwide and is defined as a glucose intolerance that develops during pregnancy and resolves post-partum (Coustan, 2013; Mack & Tomich, 2017). Pregnancies complicated by GDM have been shown to have higher concentrations of circulating EVs than uncomplicated pregnancies, and that a large percentage of these are PLAP-positive , suggesting that there may be increased pEV biogenesis and release in GDM pregnancies (Salomon, 2016). pEVs have been shown to interact with maternal organs, influencing skeletal muscle biology (Kupper & Huppertz, 2022)and contributing to insulin resistance in the mother (Kandzija *et al.*, 2019; Palma *et al.*, 2022). Whilst the importance of pEVs in the pathogenesis of GDM is of obvious importance, there is also evidence that EVs in maternal circulation, and their miRNA cargo, could potentially influence placental development and dysfunction in GDM (Kennedy *et al.*, 2019; Quilang *et al.*, 2022). Gillet et al. (2019), detected 10 maternal serum EV miRNAs that were upregulated in GDM at early gestation (6-15 weeks) (Gillet *et al.*, 2019). Whilst the functional role of these was not reported, eight of the altered miRNAs have predicted functions related to vascular development. Given that placental vascular dysfunction is a feature of GDM, it is possible that these circulating EV-enclosed miRNAs may contribute to placental dysfunction in GDM. Indeed our recent observation that vascular regulatory miRNAs are also altered in both EVs in maternal circulation, and in placenta in GDM pregnancies that go on to deliver LGA babies (Kennedy *et al.*, 2019), supports this hypothesis. Although further work is required to confirm this, a potential role for EV miRNAs in pathogenesis of GDM and associated fetal growth, could provide both novel diagnostic and therapeutic appurtuniteis to reduce clinical burden asosociated with GDM.

Extracellular vesicles in Pre-eclampsia

Pre-eclampsia (PE) is a multisystem disease characterised by new-onset hypertension after 20 weeks of gestation and one or more additional indicator of organ dysfunction, such as proteinuria (Brown *et al.*, 2018; American College of Obstetricians and Gynecologists, 2020). It's incidence ranges from two to five percent and it can result in both fetal and maternal morbidity and mortality (Huppertz, 2008; Lisonkova & Joseph, 2013; Jin & Menon, 2018). To date, pre-eclampsia is the obstetric complication in which EVs have been most extensively studied.

Several studies have suggested pEVs provide a link between placental damage and the maternal phenotype in PE. Dutta et al. demonstrated that the injection of EVs from trophoblast cultured in hypoxic (1% oxygen) conditions into the tail veins of pregnant rats increased the mean systolic blood pressure more than the administration of trophoblast derived EVs produced in normoxic (8% oxygen) conditions (Dutta *et al.*, 2020). Similarly, Han et al. found EVs from freeze-thaw injured placenta induced hypertension and proteinuria when administered to non-pregnant mice, disrupted endothelial integrity and induced vasoconstriction (Han *et al.*, 2020). Powell et al. demonstrated the uptake of sEVs from human maternal plasma into the vessel wall of mouse mesenteric arteries (Powell *et al.*, 2022). Exposure to sEVs isolated from the plasma of individuals with PE produced significantly more vasoconstriction and less endothelium-induced relaxation in these vessels than sEVs from plasma of individuals without PE.

In addition to peripheral vascular changes, pre-eclampsia is also characterised by neurological changes, manifesting as hyperreflexia and potentially seizures (eclampsia). Leon et al. found that human brain endothelial cells, used as an in vitro model of the blood-brain barrier, showed increased permeability and reduced transendothelial electrical resistance when exposed to plasma EVs from mothers with PE compared to plasma EVs from mothers without PE (Leon *et al.*, 2021). A similar response was seen to EVs from placenta cultured in hypoxic conditions (1% oxygen) compared with EVs from placenta cultured in normoxic conditions (8% oxygen) (Figure 4). These changes were mitigated by co-administration of magnesium sulphate, a drug that is used clinically to prevent seizures in pre-eclampsia.

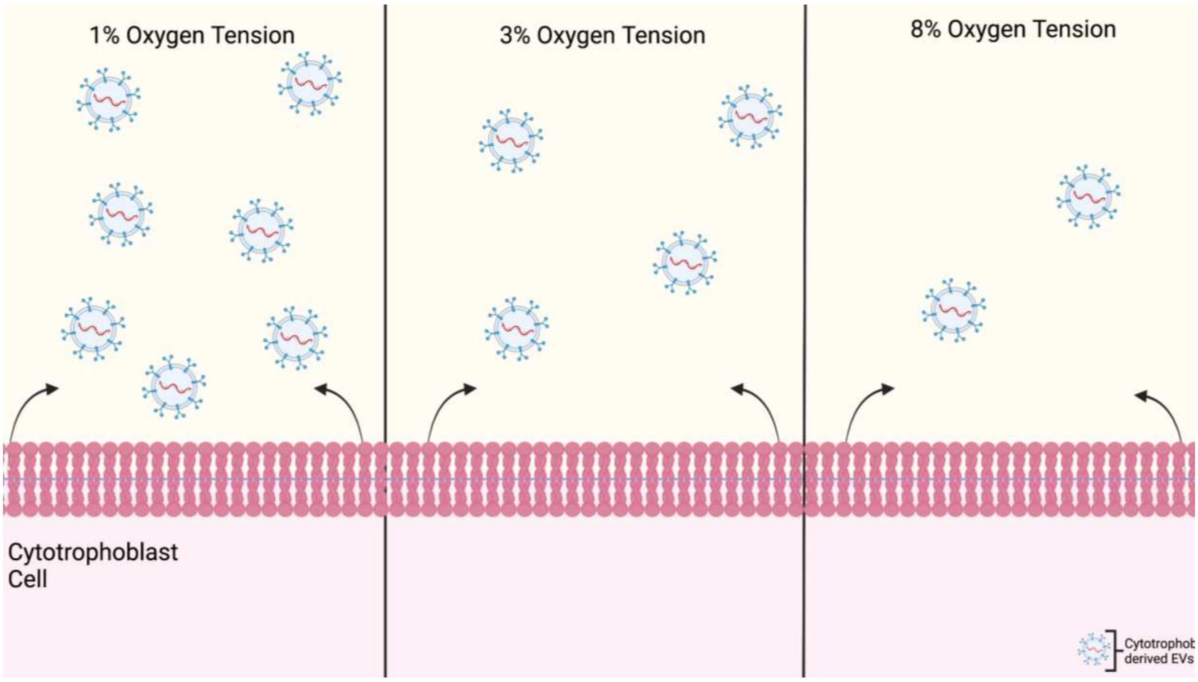


Figure 4. Oxygen tension in pEV biogenesis and bioactivity. Saloman et al. carried out a study by which they treated cytotrophoblast (CT) cell cultures with different levels of oxygen tension. They found that in the higher oxygen tensions, fewer EVs were secreted by CT cells than in the lower oxygen tensions. They hypothesised that CT derived exosomes are formed under hypoxic conditions within early pregnancy in the placenta and that this may be an adaptive response to encourage trophoblast invasion (Salomon et al., 2015). This supports the further investigation into early pregnancy pEV analysis in their role in both healthy and abnormal pregnancies (Truong et al., 2017; Dutta et al., 2020). Created in Biorender.com

While placental EVs may negatively impact maternal health in the later stages of pre-eclampsia, there is also evidence to suggest maternal EVs may negatively impact the placenta earlier in pregnancy. Kohli et al. administered EVs from serum-starved cultured mouse endothelial cells to pregnant mice at days 10.5 and 11.5 of gestation (E10.5, E11.5), the time at which the mature mouse placenta is established (Kohli *et al.*, 2016). This resulted in significant increases in maternal blood pressure, proteinuria and soluble fms-like tyrosine kinase (sflt1) compared with a control media. Fetal and placental size were reduced, fetal survival was reduced and placental inflammasome activation was increased in pregnancies administered EVs versus control media. The placentas of EV-administered mice also showed structural changes consistent with malperfusion.

In 2020 Li et al. isolated EVs from maternal plasma to quantify their concentration and size, as well as determine whether components of their cargo were up or down regulated across 60 healthy, PE and FGR pregnancies. Their findings displayed a twofold upregulation of the miRNA cargos miR-153-3p and miR-325-3p in pre-eclamptic pregnancies compared with healthy pregnancies (Figure 5) (Li *et al.*, 2020). The overexpression of miR-153 in humans is hypothesised to inhibit cell proliferation and invasion and encourage apoptosis (Zeng *et al.*, 2017). Further to this, miR-153 has also been shown to bind to the 3' untranslated region of Hypoxia Inducible Factor 1 (*HIF1*) and supresses expression. This is associated with reduced tube formation in the endothelial cells of the primary human umbilical vein, as well as reduced VEGFA expression and thus inhibited angiogenesis.

One notable finding of Li's work was that the seven miRNAs that were differentially expressed in pEVs from healthy and PE pregnancies did not show differences in miRNA sequencing of the whole plasma free miRNA. This was further shown in a study by Hromadnikova et al., in which the capacity of C19MC miRNAs to predict FGR was higher when expression was analysed from lysed circulating maternal exosomes rather than whole plasma miRNA (Hromadnikova *et al.*, 2019). Li et al. also demonstrated an association between pEV cargo and the placental transcriptome, thus providing evidence that pEVs isolated from maternal blood can act as a non-invasive placental biopsy (Tannetta *et al.*, 2017).

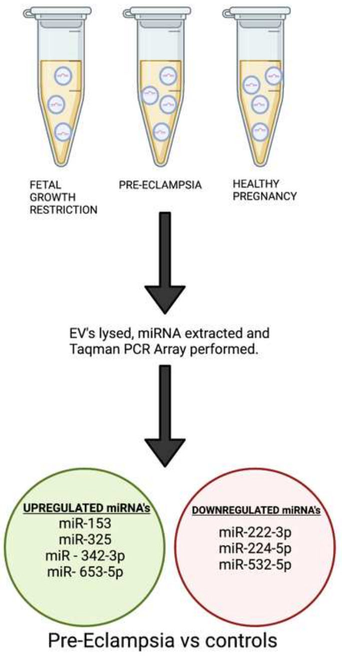


Figure 5. Summary of method and findings of Li et al. 2020. Li et al extracted maternal plasma from 60 mothers who had either a healthy pregnancy, or suffered from FGR and pre-eclampsia. They isolated EVs and lysed them to determine whether their miRNA cargo was altered between each control group and found a collection were up and downregulated in pre-eclampsia compared to the two control groups. Created in Biorender.com.

As well as contributing to the development of pre-eclampsia, EVs may have potential as therapies. One example of this is decidual mesenchymal stem/stromal cell (DMSC) EVs. DMSCs have been shown to reduce endothelial cell dysfunction in response to oxidative and inflammatory damage when co-cultured with them (Alshabibi *et al.*, 2018). Zheng et al. hypothesised that the beneficial effects of DMSCs may be mediated by EVs, which in turn could be used to mitigate the endothelial dysfunction seen in pre-eclampsia (Zheng *et al.*, 2020). This was supported by their finding of increased cell attachment and proliferation and decreased IL-6 expression and lipid peroxidation when human umbilical vein endothelial cells (HUVECs) were exposed to DMSC EVs in combination with serum from pre-eclamptic mothers compared with pre-eclamptic serum alone.

Extracellular Vesicles in preterm birth

There are multiple mechanisms that lead to the same final common pathway of preterm birth, which in turn is the leading cause of perinatal mortality and morbidity in developed countries (Goldenberg *et al.*, 2008). EVs appear to contribute to the normal initiation of labour at term (Palomares *et al.*, 2021; Yadava *et al.*, 2021), making it plausible that they could form part of the mechanistic pathway in some cases of preterm birth.

In an *in vivo* mouse study (Sheller-Miller *et al.*, 2019), maternal plasma EVs at gestational day 18 (E18, late pregnancy) were found to contain proinflammatory cargo, thought to contribute to labour and delivery. Administration of E18 EVs to pregnant mice at E15 resulted in preterm birth in four out of five dams, while none of the dams adminsitered E9 EVs delivered prematurely.

Intraperitoneally administered EVs were delivered to the uterine tissues regardless of gestational age, while E18 EVs were found to both prepare the uterus and cervix for parturition as well as promote prepartum proinflammation in fetal membranes. Whilst this is a murine model with a small sample of mice (n=15), the study highlights the importance of pEVs in paracrine signalling within pregnancies.

When studying human pregnancy, Menon et al. found fewer PLAP^{+ve} (placental) EVs in maternal plasma from the first and second trimesters of pregnancies ending in preterm birth compared with pregnancies ending in term birth (Menon *et al.*, 2020). In contrast, the number and size distribution of PLAP^{-ve} EVs was similar between the two groups. Using sequential windowed acquisition of all theoretical mass spectra (SWATH) mass spectrometry of pEV cargo, they identified 96 proteins which differed significantly across gestation between pregnancies ending in term and preterm birth. The highest scoring network for these proteins related to cell death and survival. Analysing total circulating EV miRNAs using next generation sequencing they also identified 173 miRNAs that differed significantly across gestation between pregnancies ending in term and preterm birth (Menon *et al.*, 2019). Signalling pathways targeted by these miRNAs included p53, fitting with the proteomic finding of differences relating to cell death and survival, TGF-β signalling and glucocorticoid receptor signalling.

An additional cross-sectional study by Menon et al. compared the protein cargo of total circulating EVs in maternal plasma between pregnancies at term not in labour (TNIL), at term in labour (TL), with preterm premature rupture of membranes (pPROM) and ending in preterm birth (PTB). Canonical pathway analysis of the results of SWATH mass spectrometry showed similarities between TNIL and PTB EV cargo when compared with TL EVs. Differences were evident, however, between pPROM EV and PTB EV cargo, with increased concentrations of proteins related to inflammation, coagulation activation and response to oxidative stress in PTB EVs. Individual proteins related to cholesterol signalling, cell proliferation and classical immune activation showed significant differences between the four groups on pairwise comparison (Menon et al, 2019b) (Figure 6). These findings suggest further investigation of different preterm birth phenotypes may provide greater insights than studying preterm birth as a single entity. Menon's research team makes up the majority of the recent literature in this field, highlighting the novelty of this area and the resulting literature gaps. Further weight will be added to their findings if they can be replicated by other researchers.

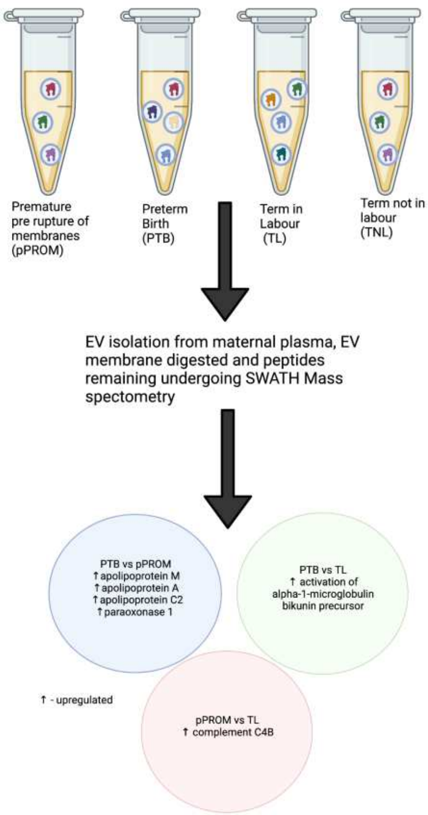


Figure 6. Summary of method and findings of Menon et al. 2019b. EVs isolated from maternal plasma were collected from 4 groups term not in labor (TNIL, n = 13); term in labor (TL, n = 11); preterm premature rupture of membranes (pPROM, n = 8); and group 4: preterm birth (PTB, n = 13). SWATH Mass spectrometry protieomics were performed displaying notable differences between the 4 groups.Created in Biorender.com

McElrath et al. displayed the potential of using EVs isolated from maternal plasma in the first trimester of singleton pregnancies as a source of predictive biomarkers for PTB before 35 weeks of gestation (McElrath *et al.*, 2019). Using a 1:2 case-controlled study, a panel of five EV proteins was selected based on analysis of samples from a training set of 135 pregnancies. When tested on a further 126 pregnancies, their marker panel had an AUC of 0.74 (95%CI 0.63-0.81), with a positive likelihood ratio of 2.70 and a negative likelihood ratio of 0.27. Zhao et al. analysed EV lipids in second trimester maternal plasma and found higher levels of PS(34:0) in microvesicles from 27 pregnancies ending before 37 weeks of gestation compared with 66 full term pregnancies (Zhao *et al.*, 2020). When tested in a validation set of a further 83 pregnancies, microvesicle PS(34:0) had an AUC of 0.71 (95% CI 0.60-0.82) for predicting birth before 37 weeks. Although both of these findings have yet to be externally validated, they support the utility of EVs as a source of predictive markers in pregnancy.

Extracellular Vesicles in FGR

There are fewer studies focussing specifically on FGR than the other obstetric conditions discussed in this review. However, there is considerable overlap, both in terms of pathophysiology and incidence, between early-onset FGR (<32 weeks of gestation) and early-onset pre-eclampsia (<34 weeks of gestation). This may mean some of the findings about EVs in pre-eclampsia may prove relevant to FGR in the future.

Miranda et al. demonstrated an association between the ratio of pEVs ($CD63^{+ve}$ and $PLAP^{+ve}$) to total circulating EVs ($CD63^{+ve}$) and fetal growth *in utero* (Miranda *et al.*, 2018b). They found the ratio of pEVs to total EVs was significantly reduced in small-for-gestational age (SGA) fetuses (EFW <10th centile) compared with appropriate-for-gestational-age (AGA) fetuses, with a further reduction in the ratio for fetuses with FGR (EFW<10th centile and abnormal Doppler studies or EFW <3rd centile). This raises the possibility of monitoring pEV quantity as a marker for placental insufficiency and thus fetal growth.

Ariyakumar et al. also found total EV concentration was significantly lower in maternal plasma from pregnancies with FGR (birth weight <10th with absent or reversed umbilical artery end-diastolic flow) than in normal pregnancies, with FGR maternal plasma EV concentrations similar to that of non-pregnant individuals (Ariyakumar *et al.*, 2021). The concentration of Fas ligand (FasL), which promotes immune tolerance through suppression of the NF-κβ subunit p65, was lower also in EVs from FGR pregnancies than AGA pregnancies. This suggests a possible pathophysiological effect of EVs in FGR.

Conclusion

Extracellular vesicles are a key mediator of cell-cell communication, carrying miRNA, proteins and surface antigens. It is perhaps unsurprising, therefore, that they appear to be involved in so many key areas of cross-talk between the mother, the placenta and the fetus. In healthy pregnancy this includes implantation, immunomodulation and the initiation of labour. In pregnancy complications it includes the production of maternal pre-eclampsia manifestations in response to EVs from hypoxic or otherwise damaged placenta.

Studying maternal, placental and fetal EVs in healthy and complicated pregnancies is providing novel insights into the causes and pathophysiology of obstetric diseases. It is also allowing researchers to identify novel biomarkers to predict which pregnancies are at higher risk of complications. In time we may be able to harness this knowledge to use EVs as a therapy, either by utilising naturally produced EVs that have beneficial effects, as in the case of DMSC EVs, or by using artificially created EVs as a drug delivery system. Given the lack of current effective treatments for many obstetric complications, this would be of great benefit to the health of pregnant people and their future children.

Alberry M & Soothill P. (2007). Management of fetal growth restriction. *Arch Dis Child Fetal Neonatal Ed* **92,** F62-67.

Alshabibi MA, Khatlani T, Abomaray FM, AlAskar AS, Kalionis B, Messaoudi SA, Khanabdali R, Alawad AO & Abumaree MH. (2018). Human decidua basalis mesenchymal stem/stromal cells protect endothelial cell functions from oxidative stress induced by hydrogen peroxide and monocytes. *Stem Cell Res Ther* **9,** 275.

American College of Obstetricians and Gynecologists. (2020). Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin Summary, Number 222. *Obstet Gynecol* **135,** 1492-1495.

Andronico F, Battaglia R, Ragusa M, Barbagallo D, Purrello M & Di Pietro C. (2019). Extracellular Vesicles in Human Oogenesis and Implantation. *Int J Mol Sci* **20**.

Ariyakumar G, Morris JM, McKelvey KJ, Ashton AW & McCracken SA. (2021). NF-κB regulation in maternal immunity during normal and IUGR pregnancies. *Sci Rep* **11,** 20971.

Arraud N, Linares R, Tan S, Gounou C, Pasquet JM, Mornet S & Brisson AR. (2014). Extracellular vesicles from blood plasma: determination of their morphology, size, phenotype and concentration. *Journal of thrombosis and haemostasis* **12,** 614-627.

Atay S, Gercel-Taylor C, Suttles J, Mor G & Taylor DD. (2011). Trophoblast-derived exosomes mediate monocyte recruitment and differentiation. *Am J Reprod Immunol* **65,** 65-77.

Balbi C, Piccoli M, Barile L, Papait A, Armirotti A, Principi E, Reverberi D, Pascucci L, Becherini P, Varesio L, Mogni M, Coviello D, Bandiera T, Pozzobon M, Cancedda R & Bollini S. (2017). First Characterization of Human Amniotic Fluid Stem Cell Extracellular Vesicles as a Powerful Paracrine Tool Endowed with Regenerative Potential: Amniotic Fluid Stem Cell Extracellular Vesicles. *Stem cells translational medicine* **6,** 1340-1355.

Bendix I, Miller SL & Winterhager E. (2020). Editorial: Causes and Consequences of Intrauterine Growth Restriction. *Front Endocrinol (Lausanne)* **11,** 205.

Boon RA & Vickers KC. (2013). Intercellular transport of microRNAs. *Arterioscler Thromb Vasc Biol* **33,** 186-192.

Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, Hall DR, Warren CE, Adoyi G, Ishaku S & International Society for the Study of Hypertension in P. (2018). The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. *Pregnancy Hypertens* **13,** 291-310.

Buca D, Bologna G, D'Amico A, Cugini S, Musca F, Febbo M, D'Arcangelo D, Buca D, Simeone P, Liberati M, Vitacolonna E, Miscia S, D'Antonio F & Lanuti P. (2020). Extracellular Vesicles in Feto-Maternal Crosstalk and Pregnancy Disorders. *Int J Mol Sci* **21**.

Burton GJ, Redman CW, Roberts JM & Moffett A. (2019). Pre-eclampsia: pathophysiology and clinical implications. *Bmj* **366,** l2381.

Cartwright JE, Fraser R, Leslie K, Wallace AE & James JL. (2010a). Remodelling at the maternal-fetal interface: relevance to human pregnancy disorders. *Reproduction* **140,** 803-813.

Cartwright JE, Fraser R, Leslie K, Wallace AE & James JL. (2010b). Remodelling at the maternal–fetal interface: relevance to human pregnancy disorders. *Reproduction (Cambridge, England)* **140,** 803-813.

Chang G, Mouillet JF, Mishima T, Chu T, Sadovsky E, Coyne CB, Parks WT, Surti U & Sadovsky Y. (2017). Expression and trafficking of placental microRNAs at the feto-maternal interface. *Faseb j* **31,** 2760-2770.

Chen K, Liang J, Qin T, Zhang Y, Chen X & Wang Z. (2022). The Role of Extracellular Vesicles in Embryo Implantation. *Front Endocrinol (Lausanne)* **13,** 809596.

Chiarello DI, Salsoso R, Toledo F, Mate A, Vazquez CM & Sobrevia L. (2018). Foetoplacental communication via extracellular vesicles in normal pregnancy and preeclampsia. *Mol Aspects Med* **60,** 69-80.

Colella M, Frerot A, Novais ARB & Baud O. (2018). Neonatal and Long-Term Consequences of Fetal Growth Restriction. *Curr Pediatr Rev* **14,** 212-218.

Condrat CE, Varlas VN, Duica F, Antoniadis P, Danila CA, Cretoiu D, Suciu N, Cretoiu SM & Voinea SC. (2021). Pregnancy-Related Extracellular Vesicles Revisited. *Int J Mol Sci* **22**.

Coustan DR. (2013). Gestational diabetes mellitus. *Clin Chem* **59,** 1310-1321.

D'Arrigo D, Roffi A, Cucchiarini M, Moretti M, Candrian C & Filardo G. (2019). Secretome and Extracellular Vesicles as New Biological Therapies for Knee Osteoarthritis: A Systematic Review. *Journal of clinical medicine* **8,** 1867.

Doyle LM & Wang MZ. (2019). Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells* **8**.

Dutta S, Lai A, Scholz-Romero K, Shiddiky MJA, Yamauchi Y, Mishra JS, Rice GE, Hyett J, Kumar S & Salomon C. (2020). Hypoxia-induced small extracellular vesicle proteins regulate proinflammatory cytokines and systemic blood pressure in pregnant rats. *Clin Sci (Lond)* **134,** 593-607.

El Andaloussi S, Mäger I, Breakefield XO & Wood MJA. (2013). Extracellular vesicles: biology and emerging therapeutic opportunities. *Nature Reviews Drug Discovery* **12,** 347-357.

Elfeky O, Longo S, Lai A, Rice GE & Salomon C. (2017). Influence of maternal BMI on the exosomal profile during gestation and their role on maternal systemic inflammation. *Placenta* **50,** 60-69.

Evans J, Rai A, Nguyen HPT, Poh QH, Elglass K, Simpson RJ, Salamonsen LA & Greening DW. (2019). Human Endometrial Extracellular Vesicles Functionally Prepare Human Trophectoderm Model for Implantation: Understanding Bidirectional Maternal-Embryo Communication. *Proteomics* **19,** e1800423.

Familari M, Cronqvist T, Masoumi Z & Hansson SR. (2017). Placenta-derived extracellular vesicles: their cargo and possible functions. *Reprod Fertil Dev* **29,** 433-447.

Ge Q, Zhou Y, Lu J, Bai Y, Xie X & Lu Z. (2014). miRNA in plasma exosome is stable under different storage conditions. *Molecules (Basel, Switzerland)* **19,** 1568-1575.

Giacomini E, Vago R, Sanchez AM, Podini P, Zarovni N, Murdica V, Rizzo R, Bortolotti D, Candiani M & Vigano P. (2017). Secretome of in vitro cultured human embryos contains extracellular vesicles that are uptaken by the maternal side. *Sci Rep* **7,** 5210.

Gillet V, Ouellet A, Stepanov Y, Rodosthenous RS, Croft EK, Brennan K, Abdelouahab N, Baccarelli A & Takser L. (2019). miRNA Profiles in Extracellular Vesicles From Serum Early in Pregnancies Complicated by Gestational Diabetes Mellitus. *J Clin Endocrinol Metab* **104,** 5157-5169.

Goldenberg RL, Culhane JF, Iams JD & Romero R. (2008). Epidemiology and causes of preterm birth. *Lancet* **371,** 75-84.

Gordijn SJ, Beune IM & Ganzevoort W. (2018). Building consensus and standards in fetal growth restriction studies. *Best Pract Res Clin Obstet Gynaecol* **49,** 117-126.

Graves M, Howse K, Pudwell J & Smith GN. (2019). Pregnancy-related cardiovascular risk indicators: Primary care approach to postpartum management and prevention of future disease. *Can Fam Physician* **65,** 883-889.

Han C, Wang C, Chen Y, Wang J, Xu X, Hilton T, Cai W, Zhao Z, Wu Y, Li K, Houck K, Liu L, Sood AK, Wu X, Xue F, Li M, Dong JF & Zhang J. (2020). Placenta-derived extracellular vesicles induce preeclampsia in mouse models. *Haematologica* **105,** 1686-1694.

Heazell A, Moll S, Jones C, Baker P & Crocker I. (2007). Formation of syncytial knots is increased by hyperoxia, hypoxia and reactive oxygen species. *Placenta* **28,** S33-S40.

Hell L, Wisgrill L, Ay C, Spittler A, Schwameis M, Jilma B, Pabinger I, Altevogt P & Thaler J. (2017). Procoagulant extracellular vesicles in amniotic fluid. *Translational research : the journal of laboratory and clinical medicine* **184,** 12-20.e11.

Holder B, Jones T, Sancho Shimizu V, Rice TF, Donaldson B, Bouqueau M, Forbes K & Kampmann B. (2016). Macrophage Exosomes Induce Placental Inflammatory Cytokines: A Novel Mode of Maternal-Placental Messaging. *Traffic* **17,** 168-178.

Holder BS, Tower CL, Forbes K, Mulla MJ, Aplin JD & Abrahams VM. (2012). Immune cell activation by trophoblast-derived microvesicles is mediated by syncytin 1. *Immunology* **136,** 184-191.

Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, Di Giannatale A, Ceder S, Singh S, Williams C, Soplop N, Uryu K, Pharmer L, King T, Bojmar L, Davies AE, Ararso Y, Zhang T, Zhang H, Hernandez J, Weiss JM, Dumont-Cole VD, Kramer K, Wexler LH, Narendran A, Schwartz GK, Healey JH, Sandstrom P, Labori KJ, Kure EH, Grandgenett PM, Hollingsworth MA, de Sousa M, Kaur S, Jain M, Mallya K, Batra SK, Jarnagin WR, Brady MS, Fodstad O, Muller V, Pantel K, Minn AJ, Bissell MJ, Garcia BA, Kang Y, Rajasekhar VK, Ghajar CM, Matei I, Peinado H, Bromberg J & Lyden D. (2015). Tumour exosome integrins determine organotropic metastasis. *Nature* **527,** 329-335.

Hromadnikova I, Dvorakova L, Kotlabova K & Krofta L. (2019). The Prediction of Gestational Hypertension, Preeclampsia and Fetal Growth Restriction via the First Trimester Screening of Plasma Exosomal C19MC microRNAs. *Int J Mol Sci* **20**.

Huppertz B. (2008). Placental Origins of Preeclampsia: Challenging the Current Hypothesis. *Hypertension (Dallas, Tex 1979)* **51,** 970-975.

Jansen CHJR, Kastelein AW, Kleinrouweler CE, van Leeuwen E, de Jong KH, Pajkrt E & van Noorden CJF. (2020). Development of placental abnormalities in location and anatomy. *Acta obstetricia et gynecologica Scandinavica* **99,** 983-993.

Jia L, Zhou X, Huang X, Xu X, Jia Y, Wu Y, Yao J, Wu Y & Wang K. (2018). Maternal and umbilical cord serum-derived exosomes enhance endothelial cell proliferation and migration. *The FASEB journal* **32,** 4534-4543.

Jiao X, Fan Z, Chen H, He P, Li Y, Zhang Q & Ke C. (2017). Serum and exosomal miR-122 and miR-199a as a biomarker to predict therapeutic efficacy of hepatitis C patients. *J Med Virol* **89,** 1597-1605.

Jin J & Menon R. (2018). Placental exosomes: A proxy to understand pregnancy complications. *Am J Reprod Immunol* **79,** e12788.

Kalra H, Drummen GP & Mathivanan S. (2016). Focus on Extracellular Vesicles: Introducing the Next Small Big Thing. *Int J Mol Sci* **17,** 170.

Kandzija N, Zhang W, Motta-Mejia C, Mhlomi V, McGowan-Downey J, James T, Cerdeira AS, Tannetta D, Sargent I, Redman CW, Bastie CC & Vatish M. (2019). Placental extracellular vesicles express active dipeptidyl peptidase IV; levels are increased in gestational diabetes mellitus. *J Extracell Vesicles* **8,** 1617000.

Kapur A, McIntyre HD, Divakar H, Di Renzo GC, Kihara AB, McAuliffe F, Hanson M, Ma RC, Hod M & Pregnancy FWGoHi. (2020). Towards a global consensus on GDM diagnosis: Light at the end of the tunnel? *Int J Gynaecol Obstet* **149,** 257-261.

Kennedy M, Cartland S, Saravanan P, Simpson N, Scott E & Forbes K. (2019). miR-1-3p and miR-133-3p are altered in maternal serum EVs and placenta in pregnancies complicated by gestational diabetes with large-for-gestational age babies. *Endocrine Abstracts*.

Keshtkar S, Azarpira N & Ghahremani MH. (2018). Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. *Stem Cell Res Ther* **9,** 63.

Kingdom J, Huppertz B, Seaward G & Kaufmann P. (2000). Development of the placental villous tree and its consequences for fetal growth. *Eur J Obstet Gynecol Reprod Biol* **92,** 35-43.

Kohli S, Ranjan S, Hoffmann J, Kashif M, Daniel EA, Al-Dabet MM, Bock F, Nazir S, Huebner H, Mertens PR, Fischer KD, Zenclussen AC, Offermanns S, Aharon A, Brenner B, Shahzad K, Ruebner M & Isermann B. (2016). Maternal extracellular vesicles and platelets promote preeclampsia via inflammasome activation in trophoblasts. *Blood* **128,** 2153-2164.

Kupper N & Huppertz B. (2022). The endogenous exposome of the pregnant mother: Placental extracellular vesicles and their effect on the maternal system. *Molecular Aspects of Medicine* **87,** 100955.

Lancaster GI & Febbraio MA. (2005). Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins. *J Biol Chem* **280,** 23349-23355.

Lee Y-T, Tran BV, Wang JJ, Liang IY, You S, Zhu Y, Agopian VG, Tseng H-R & Yang JD. (2021). The Role of Extracellular Vesicles in Disease Progression and Detection of Hepatocellular Carcinoma. *Cancers* **13,** 3076.

Lees C, Marlow N, Arabin B, Bilardo CM, Brezinka C, Derks JB, Duvekot J, Frusca T, Diemert A, Ferrazzi E, Ganzevoort W, Hecher K, Martinelli P, Ostermayer E, Papageorghiou AT, Schlembach D, Schneider KTM, Thilaganathan B, Toos T, van Wassenaer-Leemhuis A, Valcamonico A, Visser GHA & Wolf H. (2013). Perinatal morbidity and mortality in early-onset fetal growth restriction: cohort outcomes of the trial of randomized umbilical and fetal flow in Europe (TRUFFLE). *Ultrasound in obstetrics & gynecology* **42,** 400-408.

Leon J, Acurio J, Bergman L, Lopez J, Karin Wikstrom A, Torres-Vergara P, Troncoso F, Castro FO, Vatish M & Escudero C. (2021). Disruption of the Blood-Brain Barrier by Extracellular Vesicles From Preeclampsia Plasma and Hypoxic Placentae: Attenuation by Magnesium Sulfate. *Hypertension***,** HYPERTENSIONAHA12117744.

Li H, Ouyang Y, Sadovsky E, Parks WT, Chu T & Sadovsky Y. (2020). Unique microRNA Signals in Plasma Exosomes from Pregnancies Complicated by Preeclampsia. *Hypertension* **75,** 762-771.

Li T, Greenblatt EM, Shin ME, Brown TJ & Chan C. (2021). Cargo small non-coding RNAs of extracellular vesicles isolated from uterine fluid associate with endometrial receptivity and implantation success. *Fertil Steril* **115,** 1327-1336.

Lisonkova SMDP & Joseph KSMDP. (2013). Incidence of preeclampsia: risk factors and outcomes associated with early- versus late-onset disease. *American journal of obstetrics and gynecology* **209,** 544.e541-544.e512.

Lotvall J & Valadi H. (2007). Cell to Cell Signalling via Exosomes Through esRNA. *Cell adhesion & migration* **1,** 156-158.

Mack LR & Tomich PG. (2017). Gestational Diabetes: Diagnosis, Classification, and Clinical Care. *Obstet Gynecol Clin North Am* **44,** 207-217.

Malhotra A, Allison BJ, Castillo-Melendez M, Jenkin G, Polglase GR & Miller SL. (2019). Neonatal Morbidities of Fetal Growth Restriction: Pathophysiology and Impact. *Front Endocrinol (Lausanne)* **10,** 55.

Manier S, Liu CJ, Avet-Loiseau H, Park J, Shi J, Campigotto F, Salem KZ, Huynh D, Glavey SV, Rivotto B, Sacco A, Roccaro AM, Bouyssou J, Minvielle S, Moreau P, Facon T, Leleu X, Weller E, Trippa L & Ghobrial IM. (2017). Prognostic role of circulating exosomal miRNAs in multiple myeloma. *Blood* **129,** 2429-2436.

McElrath TF, Cantonwine DE, Jeyabalan A, Doss RC, Page G, Roberts JM, Brohman B, Zhang Z & Rosenblatt KP. (2019). Circulating microparticle proteins obtained in the late first trimester predict spontaneous preterm birth at less than 35 weeks' gestation: a panel validation with specific characterization by parity. *Am J Obstet Gynecol* **220,** 488.e481-488.e411.

McVey MJ & Kuebler WM. (2018). Extracellular vesicles: biomarkers and regulators of vascular function during extracorporeal circulation. *Oncotarget* **9,** 37229-37251.

Menon R, Debnath C, Lai A, Guanzon D, Bhatnagar S, Kshetrapal P, Sheller-Miller S & Salomon C. (2020). Protein Profile Changes in Circulating Placental Extracellular Vesicles in Term and Preterm Births: A Longitudinal Study. *Endocrinology* **161**.

Menon R, Debnath C, Lai A, Guanzon D, Bhatnagar S, Kshetrapal PK, Sheller-Miller S & Salomon C. (2019). Circulating Exosomal miRNA Profile During Term and Preterm Birth Pregnancies: A Longitudinal Study. *Endocrinology* **160,** 249-275.

Merino-Gonzalez C, Zuniga FA, Escudero C, Ormazabal V, Reyes C, Nova-Lamperti E, Salomon C & Aguayo C. (2016). Mesenchymal Stem Cell-Derived Extracellular Vesicles Promote Angiogenesis: Potencial Clinical Application. *Front Physiol* **7,** 24.

Miranda J, Paules C, Nair S, Lai A, Palma C, Scholz-Romero K, Rice GE, Gratacos E, Crispi F & Salomon C. (2018a). Placental exosomes profile in maternal and fetal circulation in intrauterine growth restriction - Liquid biopsies to monitoring fetal growth. *Placenta* **64,** 34-43.

Miranda J, Paules C, Nair S, Lai A, Palma C, Scholz-Romero K, Rice GE, Gratacos E, Crispi F & Salomon C. (2018b). Placental exosomes profile in maternal and fetal circulation in intrauterine growth restriction - Liquid biopsies to monitoring fetal growth. *Placenta (Eastbourne)* **64,** 34-43.

Mishra A, Ashary N, Sharma R & Modi D. (2021). Extracellular vesicles in embryo implantation and disorders of the endometrium. *Am J Reprod Immunol* **85,** e13360.

Morelli AE & Sadovsky Y. (2022). Extracellular vesicles and immune response during pregnancy: A balancing act. *Immunol Rev*.

National Institute for Health and Care Excellence. (2015 (updated 2020)). Nice Guideline. Diabetes in pregnancy: management from preconception to the postnatal period. National Institute for Health and Care Excellence.

Out HJ, Kooijman CD, Bruinse HW & Derksen RH. (1991). Histopathological findings in placentae from patients with intra-uterine fetal death and anti-phospholipid antibodies.

European Journal of Obstetrics & Gynecology and Reproductive Biology **41,** 179-186.

Palma C, McIntyre HD & Salomon C. (2022). Extracellular Vesicles-New Players in Cell-to-Cell Communication in Gestational Diabetes Mellitus. *Biomedicines* **10**.

Palomares KT, Parobchak N, Ithier MC, Aleksunes LM, Castano PM, So M, Faro R, Heller D, Wang B & Rosen T. (2021). Fetal Exosomal Platelet-activating Factor Triggers Functional Progesterone Withdrawal in Human Placenta. *Reprod Sci* **28,** 252-262.

Paquette AG, Chu T, Wu X, Wang K, Price ND & Sadovsky Y. (2018). Distinct communication patterns of trophoblastic miRNA among the maternal-placental-fetal compartments. *Placenta* **72-73,** 28-35.

Patton AL, McCallie B, Parks JC, Schoolcraft WB & Katz-Jaffe M. (2015). Exosome bound microRNAs transcriptionally regulate embryo-endometrial dialogue impacting implantation potential for AMA patients. *Fertility and sterility* **104,** e308-e308.

Pezzana C, Agnely F, Bochot A, Siepmann J & Menasché P. (2021). Extracellular Vesicles and Biomaterial Design: New Therapies for Cardiac Repair. *Trends in molecular medicine* **27,** 231-247.

Powell JS, Gandley RE, Lackner E, Dolinish A, Ouyang Y, Powers RW, Morelli AE, Hubel CA & Sadovsky Y. (2022). Small extracellular vesicles from plasma of women with preeclampsia increase myogenic tone and decrease endothelium-dependent relaxation of mouse mesenteric arteries. *Pregnancy Hypertens* **28,** 66-73.

Quilang R, Godinho E, Timms K, Scott EM & Forbes K. (2022). ODP434 Maternally-Derived Pancreatic Extracellular Vesicle Encompassed miRNAs Influence Placental Development in Pregnancies Complicated by Gestational Diabetes. *J Endocr Soc* **6,** A671-672.

Quintanilla Rodriguez BS & Mahdy H. (2022). Gestational Diabetes. In *StatPearls*. StatPearls Publishing

Copyright © 2022, StatPearls Publishing LLC., Treasure Island (FL).

Redman CW, Tannetta DS, Dragovic RA, Gardiner C, Southcombe JH, Collett GP & Sargent IL. (2012). Review: Does size matter? Placental debris and the pathophysiology of pre-eclampsia. *Placenta* **33 Suppl,** S48-54.

Redman CWG & Sargent IL. (2008). Circulating Microparticles in Normal Pregnancy and Pre-Eclampsia. *Placenta* **29,** 73-77.

Sáez T, de Vos P, Sobrevia L & Faas MM. (2018). Is there a role for exosomes in foetoplacental endothelial dysfunction in gestational diabetes mellitus? *Placenta* **61,** 48-54.

Salomon C, Scholz-Romero K, Kobayashi M, Smith M, Duncombe G, llanes S, Mitchell MD & Rice GE. (2015). Oxygen tension regulates glucose-induced biogenesis and release of different subpopulations of exosome vesicles from trophoblast cells: A gestational age profile of placental exosomes in maternal plasma with gestational diabetes mellitus. *Placenta (Eastbourne)* **36,** 488-488.

Salomon C, Torres MJ, Kobayashi M, Scholz-Romero K, Sobrevia L, Dobierzewska A, Illanes SE, Mitchell MD & Rice GE. (2014a). A gestational profile of placental exosomes in maternal plasma and their effects on endothelial cell migration. *PLoS One* **9,** e98667.

Salomon C, Yee S, Scholz-Romero K, Kobayashi M, Vaswani K, Kvaskoff D, Illanes SE, Mitchell MD & Rice GE. (2014b). Extravillous trophoblast cells-derived exosomes promote vascular smooth muscle cell migration. *Front Pharmacol* **5,** 175.

Schumacher A, Sharkey DJ, Robertson SA & Zenclussen AC. (2018). Immune Cells at the Fetomaternal Interface: How the Microenvironment Modulates Immune Cells To Foster Fetal Development. *J Immunol* **201,** 325-334.

Sheller-Miller S, Trivedi J, Yellon SM & Menon R. (2019). Exosomes Cause Preterm Birth in Mice: Evidence for Paracrine Signaling in Pregnancy. *Scientific Reports* **9,** 608.

Simeone P, Bologna G, Lanuti P, Pierdomenico L, Guagnano MT, Pieragostino D, Del Boccio P, Vergara D, Marchisio M, Miscia S & Mariani-Costantini R. (2020). Extracellular Vesicles as Signaling Mediators and Disease Biomarkers across Biological Barriers. *International journal of molecular sciences* **21,** 2514.

Simionescu N, Zonda R, Petrovici AR & Georgescu A. (2021). The Multifaceted Role of Extracellular Vesicles in Glioblastoma: microRNA Nanocarriers for Disease Progression and Gene Therapy. *Pharmaceutics* **13,** 988.

Skotland T, Sandvig K & Llorente A. (2017). Lipids in exosomes: Current knowledge and the way forward. *Prog Lipid Res* **66,** 30-41.

Tannetta D, Collett G, Vatish M, Redman C & Sargent I. (2017). Syncytiotrophoblast extracellular vesicles - Circulating biopsies reflecting placental health. *Placenta* **52,** 134-138.

Théry C, Witwer KW, Aikawa E, Andriantsitohaina R, Baharvand H, Bauer NN, Baxter AA, Beckham C, Bielska E, Boireau W, Bongiovanni A, Brisson A, Broekman MLD, Bryl-Górecka P, Buch S, Bussolati B, Caruso S, Clayton A, Cocucci E, de Candia P, Demaret T, Dourado MR, Falcón-Pérez JM, Försönits A, Frelet-Barrand A, Fuhrmann G, Gabrielsson S, Gámez-Valero A, Gaudin R, Handberg A, Hegyesi H, Hendrix A, Hill AF, Inal JM, Ivanova A, Jackson HK, Jayachandran M, Jiang L, Kalluri R, Kaur S, Kierulf P, Kim KP, Krasemann S, Kurochkin IV, Langevin SM, Lázaro-Ibáñez E, Lee YXF, Libregts SF, Linē A, Linnemannstöns K, Lombard CA, Lukomska B, Marcilla A, Mathivanan S, McVey MJ, Mertens I, Morhayim J, Mullier F, Nawaz M, Nimrichter L, Noren Hooten N, Olivier M, Ortiz LA, Ostrowski M, Pegtel DM, Perut F, Polakovicova I, Pulliam L, Regev-Rudzki N, Rouschop KMA, Rughetti A, Saá P, Saul MJ, Schøyen TH, Shahaj E, Sharma S, Shekari F, Siljander PRM, Skowronek A, Soares RP, Stoorvogel W, Stott SL, Tixeira R, Toh WS, Tomasini R, van Herwijnen MJC, van Niel G, van Wijnen AJ, Vasconcelos MH, Veit TD, Viñas JL, Visnovitz T, Wehman AM, Weiss DJ, Wheelock AM, Xagorari A, Xander P, Zhang J-y, Zheutlin AR & Zickler AM. (2018). Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. In *J Extracell Vesicles*, pp. 1535750-n/a. Taylor & Francis, United States.

Timms K, Holder B, Day A, Mclaughlin J, Forbes KA & Westwood M. (2022). Watermelon-Derived Extracellular Vesicles Influence Human Ex Vivo Placental Cell Behavior by Altering Intestinal Secretions. *Molecular Nutrition & Food Research* **66,** 2200013.

Tong M & Chamley LW. (2015). Placental extracellular vesicles and feto-maternal communication. *Cold Spring Harb Perspect Med* **5,** a023028.

Tong M, Stanley JL, Chen Q, James JL, Stone PR & Chamley LW. (2017). Placental Nano-vesicles Target to Specific Organs and Modulate Vascular Tone In Vivo. *Hum Reprod* **32,** 2188-2198.

Truong G, Guanzon D, Kinhal V, Elfeky O, Lai A, Longo S, Nuzhat Z, Palma C, Scholz-Romero K, Menon R, Mol BW, Rice GE & Salomon C. (2017). Oxygen tension regulates the miRNA profile and bioactivity of exosomes released from extravillous trophoblast cells - Liquid biopsies for monitoring complications of pregnancy. *PloS one* **12,** e0174514-e0174514.

Umapathy A, Chamley LW & James JL. (2019). Reconciling the distinct roles of angiogenic/anti-angiogenic factors in the placenta and maternal circulation of normal and pathological pregnancies. *Angiogenesis*.

Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ & Lötvall JO. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* **9,** 654-659.

Warrander LK, Batra G, Bernatavicius G, Greenwood SL, Dutton P, Jones RL, Sibley CP & Heazell AE. (2012). Maternal perception of reduced fetal movements is associated with altered placental structure and function. *PloS one* **7,** e34851.

Wickman G, Julian L & Olson MF. (2012). How apoptotic cells aid in the removal of their own cold dead bodies. *Cell Death Differ* **19,** 735-742.

Williams PJ, Bulmer JN, Searle RF, Innes BA & Robson SC. (2009). Altered decidual leucocyte populations in the placental bed in pre-eclampsia and foetal growth restriction: a comparison with late normal pregnancy. *Reproduction (Cambridge, England)* **138,** 177-184.

Yadava SM, Feng A, Parobchak N, Wang B & Rosen T. (2021). miR-15b-5p promotes expression of proinflammatory cytokines in human placenta by inhibiting Apelin signaling pathway. *Placenta* **104,** 8-15.

Yáñez-Mó M, Siljander PRM, Andreu Z, Bedina Zavec A, Borràs FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, Colás E, Cordeiro-da Silva A, Fais S, Falcon-Perez JM, Ghobrial IM, Giebel B, Gimona M, Graner M, Gursel I, Gursel M, Heegaard NHH, Hendrix A, Kierulf P, Kokubun K, Kosanovic M, Kralj-Iglic V, Krämer-Albers E-M, Laitinen S, Lässer C, Lener T, Ligeti E, Linē A, Lipps G, Llorente A, Lötvall J, Manček-Keber M, Marcilla A, Mittelbrunn M, Nazarenko I, Nolte-'t Hoen ENM, Nyman TA, O'Driscoll L, Olivan M, Oliveira C, Pállinger É, del Portillo HA, Reventós J, Rigau M, Rohde E, Sammar M, Sánchez-Madrid F, Santarém N, Schallmoser K, Stampe Ostenfeld M, Stoorvogel W, Stukelj R, Van der Grein SG, Helena Vasconcelos M, Wauben MHM & De Wever O. (2015). Biological properties of extracellular vesicles and their physiological functions. *Journal of extracellular vesicles* **4,** 27066-n/a.

Zeng HF, Yan S & Wu SF. (2017). MicroRNA-153-3p suppress cell proliferation and invasion by targeting SNAI1 in melanoma. *Biochemical and biophysical research communications* **487,** 140-145.

Zhang J, Li H, Fan B, Xu W & Zhang X. (2020). Extracellular vesicles in normal pregnancy and pregnancy-related diseases. *Journal of cellular and molecular medicine* **24,** 4377-4388.

Zhao Q, Ma Z, Wang X, Liang M, Wang W, Su F, Yang H, Gao Y & Ren Y. (2020). Lipidomic Biomarkers of Extracellular Vesicles for the Prediction of Preterm Birth in the Early Second Trimester. *J Proteome Res* **19,** 4104-4113.

Zhao Y & Yang G. (2021). Potential of extracellular vesicles in the Parkinson's disease – Pathological mediators and biomarkers. *Neurochemistry international* **144,** 104974-104974.

Zheng S, Shi A, Hill S, Grant C, Kokkinos MI, Murthi P, Georgiou HM, Brennecke SP & Kalionis B. (2020). Decidual mesenchymal stem/stromal cell-derived extracellular vesicles ameliorate endothelial cell proliferation, inflammation, and oxidative stress in a cell culture model of preeclampsia. *Pregnancy Hypertens* **22,** 37-46.