# Extracellular vesicles in gestational diabetes mellitus: A scoping review

**Tanvi Bathla[1], Akram Abolbaghaei[1], Agafe Bless Reyes[1] and Dylan Burger[1,2]** ⓘ

## Abstract

Gestational diabetes mellitus (GDM) is one of the most common complications of pregnancy worldwide. Despite extensive study, the molecular mechanisms leading to GDM and associated perinatal complications are not well understood. The condition is also associated with an increased risk of future cardiometabolic disease in both mothers and their offspring. Thus, there is a pressing need for the development of effective screening tools and to identify novel molecular mechanisms responsible for the short and long-term risks associated with GDM. In this regard, extracellular vesicles (EVs) offer promise as novel biomarkers of GDM-mediated changes to both mother and fetus. The purpose of this scoping review is to provide an overview of studies examining EVs in the context of GDM. EMBASE and Ovid Medline were searched for articles published from inception to December 2020. We update current knowledge in this area and identify key knowledge gaps with recommendations for future research.

## Keywords

Gestational diabetes, pregnancy, fetus, placenta, exosomes, microvesicles, miRNAs

## Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance that is diagnosed for the first-time during pregnancy.[1] GDM is recognized as one of the most common complications of pregnancy with a worldwide prevalence of ∼6–13% depending on region and the availability of maternal care.[2] The timing of onset of GDM can vary during pregnancy but it is typically diagnosed at 24–28 weeks of gestation. Early diagnosis and management of GDM is a matter of great interest because of the associated adverse short and long-term maternal and fetal outcomes. During pregnancy, mothers with GDM have increased risk of developing pre-eclampsia, post-partum hemorrhage, and higher incidence of operative delivery.[3] There is also a high likelihood of recurrence of GDM in a subsequent pregnancy (up to 48%).[4]

Although glucose tolerance typically returns to normal in the postpartum period, women with GDM have a 20–70% risk of developing type 2 diabetes in the first decade after delivery.[5] Moreover, recent studies have also demonstrated a relationship between GDM and later-life cardiovascular disease. Women with GDM have a two-fold higher risk of developing cardiovascular disease independent of the intercurrent development of type 2 diabetes.[6] Post-delivery, mothers impacted by GDM are also at increased risk of developing renal disease.[7] GDM also has a significant impact on short and long-term outcomes in offspring. There is an increased risk of fetal macrosomia which in turn is associated with a higher incidence of shoulder dystocia and birth trauma.[8] Maternal GDM poses an increased risk of hypoglycaemia and respiratory distress in the newborn infant which in turn is

[1]Kidney Research Centre, Ottawa Hospital Research Institute, Ottawa, ON, Canada
[2]Department Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada

**Corresponding author:**
Dylan Burger, Kidney Research Centre, Ottawa Hospital Research Institute and Department of Cellular and Molecular Medicine, University of Ottawa, 2513-/451 Smyth Road, Ottawa, ON K1H 8M5, Canada.
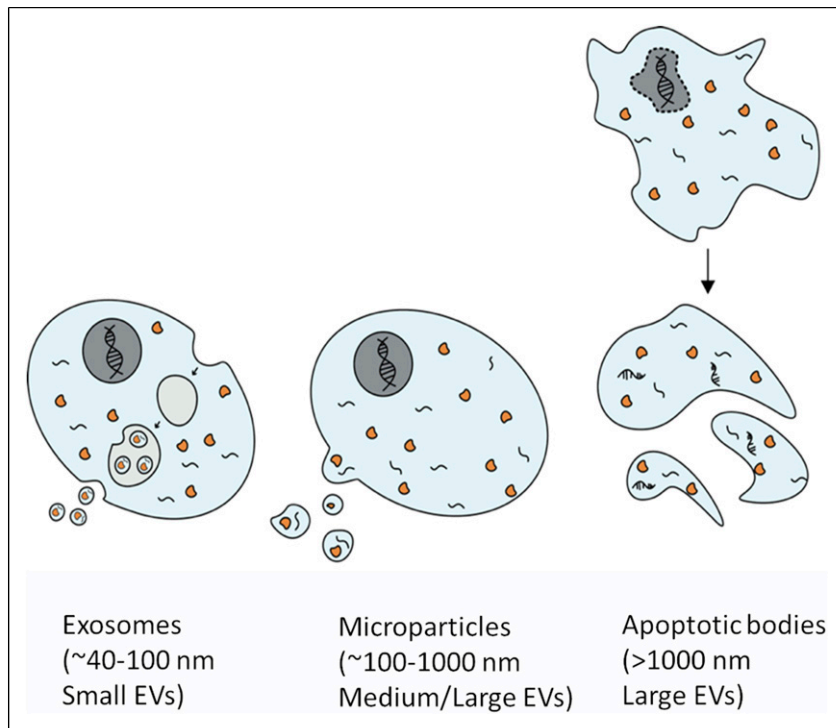Email: dburger@uottawa.ca

**Figure 1.** Biogenesis of extracellular vesicle (EV) subtypes.

associated with increased incidence of neonatal ICU admission.[9] Post-delivery, children born to mothers affected by GDM have increased lifetime risk of obesity, metabolic syndrome and cardiovascular disease.[10]

Although the prevalence of GDM is increasing,[11] our understanding of the underlying cellular mechanisms responsible for its development remain incompletely understood and are a matter of active investigation. Recent studies have shown that extracellular vesicles (EVs) can provide novel insights into GDM. Extracellular vehicles (EVs) are membrane-encapsulated particles released by cells into their extracellular environment during physiological conditions as well as stress, injury, or death.[12,13] Once released, EVs can accumulate in biological fluids including blood, cerebrospinal fluid, tears, urine, and ascites fluid.[13] In the context of pregnancy, EVs from both mother and fetus can be found in maternal plasma. Indeed, EVs released from placenta are seen in maternal circulation as early as 6 weeks and their concentration increase as gestation advances.[14]

Historically, EVs have been subclassified based on cellular origin, function and size with the most prominent subclasses being exosomes, apoptotic bodies and microparticles (MPs).[12] Exosomes, which range from 40 to 100 nm in size, exist possibly in all biological fluids.[12,15] They are characterized by the presence of tumor susceptibility gene 101 (TSG101), endosomal sorting proteins, membrane transport and fusion proteins and tetraspanins

(CD63, CD81, and CD9).[16] They do not appear to contain nuclear material and play a crucial role in intracellular communication[12,17] By contrast, apoptotic bodies are the end product of cell shrinkage and fragmentation during apoptotic cell death.[12,18] Cell organelles, nuclear material, and protein are all present in apoptotic bodies which are rapidly removed by phagocytosis after their release in vivo[12,18]. They are greater than 1000 nm in size and appear to exert anti-inflammatory effects.[12,18] Microparticles/microvesicles are intermediate sized EVs (~100–1000 nm) that are released from stressed cells and contain miRNA, mRNA and membrane and cytosolic protein but appear to lack nuclear material.[12,18] Figure 1 shows the biogenesis of major EV subtypes. While the various EV subpopulations differ in both size and biogenesis, there is considerable overlap, and most isolates are heterogeneous in nature. Thus, the International Society of Extracellular Vesicles advocates for more inclusive nomenclature with ~100–1000 nm vesicles termed "medium/large EVs" (L-EVs) and ~40–100 nm vesicles termed "small EVs" (S-EVs).[19] We will therefore use these terms throughout the remainder of this review.

Evidence from our laboratory and several others suggests that the diabetic environment can alter the formation of EVs. For example, high glucose stimulates release of L-EVs from endothelial cells,[20] podocytes[21] and platelets.[22] In vivo, levels of circulating[23,24] and urinary[21,25] EV levels are increased in animal and human diabetes and may have prognostic value. In this regard, we recently reported that

high levels of circulating endothelial-derived EVs are associated with adverse pregnancy outcomes in type 1 diabetes.[26] Diabetes also appears to impact on the molecular composition of EVs. Jansen *et al.* showed that the miRNA species incorporated into L-EVs are altered in diabetic subjects and in endothelial cells exposed to high glucose.[27] Similarly, we have shown that the proteomic composition of L-EVs is altered when formed under high glucose conditions.[20]

Given the growing interest in the impact of GDM on EVs during and after pregnancy we conducted a scoping review to examine the effect of GDM on EV formation as well as miRNA and protein composition. We discuss the relationship between changes to EV formation/composition and maternal and fetal outcomes along with recommendations for future research.

## Search strategy, design and eligibility

A structured search strategy was developed to identify publications assessing extracellular vesicles in GDM. Publications included had to meet the following requirements: (1) involve the study of EVs (or microparticles/ectosomes/microvesicles as defined in the introduction), (2) involve the study of human or animal models of GDM. Narrative reviews, conferences abstracts, studies not related to pregnancy or diseases other than diabetes were also excluded.

Electronic searches of Ovid MEDLINE <1946-December 04 2020>, and Embase Classic+Embase <1947-2020 December 07>, were conducted using the search strategy presented in Appendix S1. The initial search identified a total of 169 citations (Medline and Embase). After removing duplicate citations and limiting to studies in English, 168 citations were selected for further investigations. After evaluation of abstract and removing review and conference papers, 64 citations were selected for full review. Two independent reviewers (TB and AA) reviewed the 64 publications to identify those that met the inclusion criteria. In cases where there were discrepancies, a final consensus (for inclusion) was reached with the assistance of a third independent reviewer (DB). In total, 18 publications were included for this review

## Results

### Changes in EV levels and physicochemical properties in GDM

Changes in EV formation are commonly seen in cardiovascular conditions including diabetes and this can be reflected in altered levels in various biofluids.[28] Depending on the source of the EVs studied (i.e., plasma vs urine or other biofluids), such changes may be reflective of diabetic complications including endothelial injury, kidney damage, or, in the context of pregnancy, placental stress.[29] Our search identified eight studies that describe changes to the quantity of EVs in the context of GDM.

Salomon et al.[30] examined the concentration of circulating S-EVs from maternal circulation and placental origins in GDM as compared to normal pregnancy. They assessed both CD63 (total EVs) and placental alkaline phosphatase (PLAP, placental S-EVs) by an ELISA-based approach and showed that the plasma concentration of both populations increased in pregnancy and with advancing gestation. Interestingly, the concentration of total S-EVs in early gestation was two-fold higher in women who subsequently developed GDM as compared to normoglycaemic pregnancy. When assessing placental EVs, the amount of PLAP per S-EV was found to be lower in GDM as compared to normal pregnancy in early gestation, even though both total S-EVs and placental S-EVs were higher. This might be due to an increased secretion of S-EVs from non-placental sources. Functionally, EVs in maternal plasma from GDM exerted pro-inflammatory effects on endothelial cells by releasing cytokines[30] which may influence maternal and fetoplacental homeostasis.

The above findings are supported by Arias et al.[31] who also showed that circulating EV levels increased significantly with the progression of pregnancy with levels 1.8 and 2.3 times higher in second and third trimester respectively as compared to first trimester. They isolated S-EVs using the ExoQuick reagent and concentration was determined by nanoparticle tracking analysis. Pregnant women who subsequently developed GDM exhibited higher levels of EVs in the first trimester.

James-Allan et al.[32] also showed that the plasma concentration of S-EVs was significantly higher in pregnant individuals as compared to non-pregnant group and that levels were further elevated in the GDM cohort. In particular, they found that levels of placenta derived S-EVs were significantly higher in GDM as compared to normal pregnancy group.

Franzago et al.[33] performed a comprehensive assessment of circulating EV levels using flow cytometry focusing on leukocyte-derived (IEVs), endothelial derived (eEVs), platelet derived EVs (pEVs) and circulating adipocyte derived EVs (aEVs). In contrast to the above studies, they did not observe numeric differences in levels of EV subpopulations between GDM and normal pregnancy. However, flow cytometry-based approaches to EV quantification are generally focused on lEVs given limitations in sensitivities of the instrument. Thus, there may be differences in the formation of EV subtypes in GDM. Interestingly, the authors observed that aEV levels expressed as percentage of total EVs was higher in controls as compared to GDM.

There is some debate as to the effect of GDM on the physical properties of EVs. On the one hand, Arias et al.[31]

reported no significant differences in the size distribution of circulating EVs between GDM and normal glucose tolerance. Conversely, James-Allan et al.[32] showed that the mean size of total circulating S-EVs was higher in the non-pregnant group as compared to normal glucose tolerant and GDM group. This difference could be attributed to the fact that James-Allan et al.[32] compared the size between GDM and normal pregnancy while Arias et al.[31] compared it with a non-pregnant group. Indeed, both reported no difference in sizes between GDM and normal pregnancy group.

Particle size has also been assessed in EVs isolated from umbilical cord blood by Cao et al.[34] with both size of EVs and concentration of EVs significantly higher in GDM compared with normoglycemia. However, while both Arias et al.[31] and James-Allan et al.[32] measured EV size in different trimesters, Cao et al.[34] measured in umbilical blood after delivery, which might explain the divergence.

*Impact of GDM on properties of EV from other sources.* While plasma is the most commonly studied source of EVs, EVs from other sources may also provide insights into the impact of GDM. In this regard, Monteiro et al.[35] isolated L-EVs from gingival crevicular fluid at 11–14 weeks of gestation from GDM and normal pregnancies using Exo-Quick. Nanoparticle tracking analysis demonstrated that the mean concentration of L-EVs was significantly higher in GCF obtained from pre-symptomatic GDM women as compared to normal pregnancies. These data support the trend seen with the circulating S-EVs discussed previously. The authors postulated that in GDM, a hyperglycemic and pro-inflammatory state stimulates the release of EVs in oral fluids in early pregnancy. The increase in EV release in GDM is also found in isolated omental adipose tissue. Jayabalan et al.[36] found that the concentration of S-EVs isolated from human omental adipose tissue was significantly higher in GDM as compared to normal pregnancies.

Finally, Saez et al.[37] used high glucose treatment of HUVEC (Human umbilical vein endothelial cells) to model GDM. They postulated that high glucose enhanced the release of S-EVs, though there was no change in their size as compared to normal glucose concentration S-EVs. This is consistent with our work which also showed increases in EV release from cultured endothelial cells following high glucose exposure, although we also observed increases in the size of these EVs.[20]

## Changes in EV miRNA IN GDM

EVs contain a large variety of molecules including proteins, lipids, and nucleic acids that are generally reflective of their cell of origin. miRNAs are of particular interest in EV analysis. For one thing, their small size (∼20–25 nucleotides in their mature form) is compatible with incorporation into submicron vesicles. In fact, there is evidence of intracellular machinery which mediates insertion of miRNA into EVs.[38] Moreover, miRNA play a significant role in regulation of gene expression (estimates suggest that miRNA controls about 60% of all protein-coding genes) and thus may provide insights into the health status of the cell of origin. In the context of GDM, changes to the miRNA composition in EVs may be indicative of maternal or placental alterations and are therefore of significant interest for advancing understanding of disease pathogenesis. In our review, we identified five reports that examined miRNA in the context of GDM (Figure 2).

Gillet et al.[39] studied difference in expression of circulating EV- miRNA and "placenta specific" miRNAs in EVs from GDM and normal pregnancies They collected blood samples at 6 and 15 weeks of gestation using a candidate-based approach they examined levels of 17 miRNAs found to be associated with adverse outcomes in pregnancy. They examined this miRNA panel in serum EVs from pregnant women who later developed GDM compared with women with normal pregnancies. They observed increases in levels of 10/17 candidate miRNAs in the GDM group compared with their matched controls (Figure 2). Using Ingenuity pathway analysis, the authors examined involvement of differentially expressed miRNA in molecular processes. They reported that five upregulated miRNAs (miR–122-5p, 132-3p, 29b-3p, 182-3p, and 29a-3p) were involved in regulation of glucose homeostasis and insulin secretion. Finally, to determine whether expression of EV miRNA species was associated with placental alterations they examined associations between miRNA level and placental ultrasonography measurements at 11–13 weeks of gestation. They found weak associations between miR-517-5P with placental height and miR-136-5p with placental height and estimated placental volume. The main strengths of this study are selection approach thus reducing confounding bias, fair rationale for the candidate miRNA selected, association with clinical variables and their attempt to link miRNA to molecular pathways involved in GDM. However, there are certain weaknesses. For example, the candidate-based approach to miRNA analysis is biased and might have missed some important miRNA that are altered. It is also unclear whether EVs were from placental or maternal circulation and there was no validation of molecular targets.

miRNA alterations may also be seen in urinary EVs. A recent study by Herrera-Van Oostdam[40] examined the expression of S-EVs miRNA purified from urine in the 1st, 2nd, and 3rd trimester of pregnancy. Using a candidate-based approach they assessed differences in between GDM and healthy pregnancy. They focused on five miRNA species: miR-222-3p and 16-5p and the "trophoblastic miRs" 516-5p, 517-3p, 518-5p. These miRNAs had previously been implicated in diabetic injury. There were no significant differences in urinary EV expression of the five
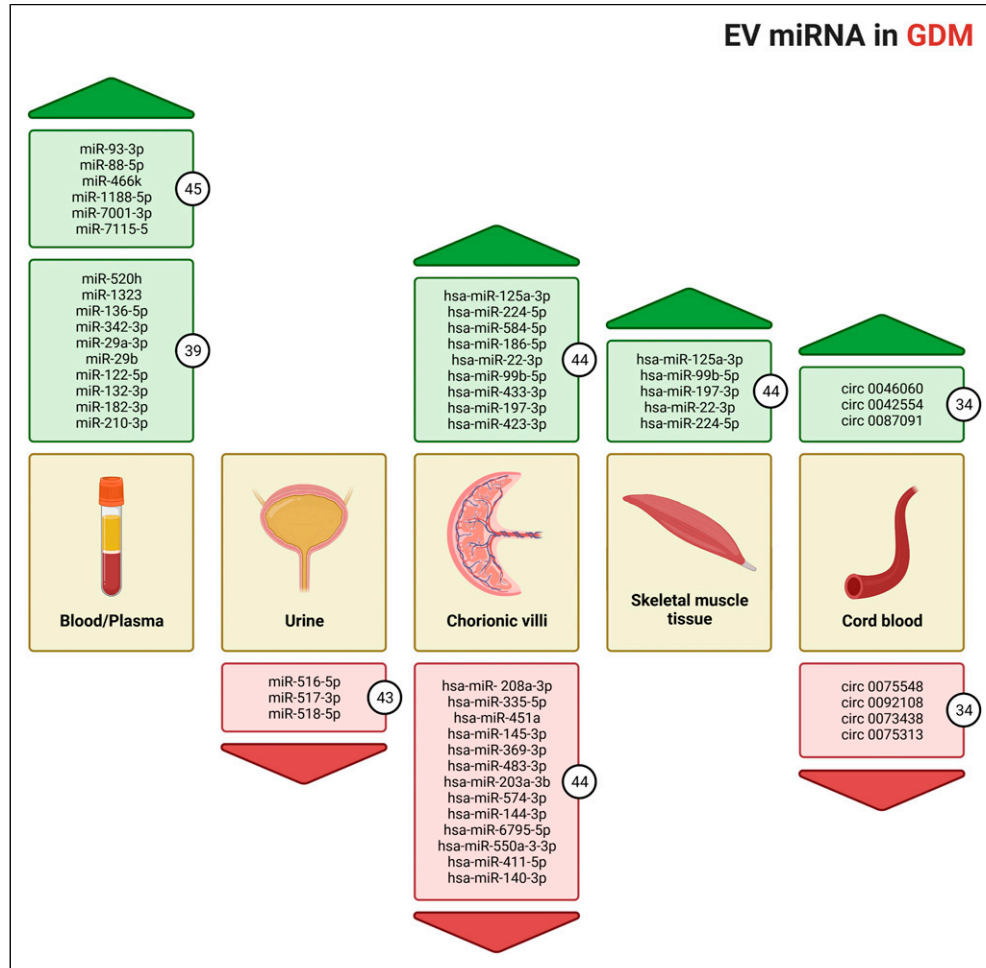
**Figure 2.** EV-miRNA species alterations in GDM. Shown are miRNA species that are increased (green) or decreased (red) in the context of GDM. White circles indicate the reference number for individual studies. Created with BioRender.com.

analyzed miRNAs between healthy pregnancy and GDM in the 1st trimester. However, the expression of miR-222-3p in the placental S-EVs from healthy women increased significantly as the pregnancy progressed. In GDM, the pattern was more complex with modest increases from 1st to 2nd trimester but significant decreases in the 3rd trimester. Similarly, when examining the "trophomiRs" they reported an increase in healthy women as the pregnancy progressed, from the first to the third trimester. In the GDM group, the expression increased from the 1st to the 2nd trimester, but a marked decrease was observed in the 3rd trimester and the expression levels of the three miRNAs were significantly downregulated as compared to the healthy pregnancy group. In case of miR-16-5p, the authors found that its expression was only detectable in S-EVs purified from patients with GDM in second trimester, whereas it was undetectable in the healthy population. Using target prediction and bioinformatic tools the authors reported that these five miRNAs could together regulate

more than 100 genes linked to a variety of biological processes including biosynthesis of fatty acids and insulin signaling.

Nair et al.[41] conducted an unbiased screen of miRNA in chorionic villi explants and their released S-EVs by next generation sequencing. They found distinct miRNAs profiles in GDM chorionic villi as compared to normal pregnancy. When examining miRNA in explant-derived S-EVs nine miRNAs were found to be significantly up-regulated and fourteen miRNAs were significantly down-regulated (Figure 2). The authors also noted several differences in miRNA abundance between EV miRNA levels compared with their cell of origin suggesting selective packaging of certain miRNA into EVs. Interestingly, five candidate miRNAs (miR-125a-3p, 99b-5p, 197-3p, 22-3p, and 224-5p) were also up-regulated in skeletal muscle tissue from GDM pregnancies. The authors concluded that this is due to specific targeting of chorionic villi EVs to skeletal muscle and delivery of miRNA content. In support of this

they showed that villi-derived EVs modulate the response of skeletal muscle cells to insulin in culture.

EV miRNA profiling has also been used to assess molecular alterations associated with GDM intervention. Xiao et al.[42] treated mice with a polysaccharide from *Lycium barbarum*, a Chinese herb that has been shown to improve lipid metabolism in diabetes. They examined the miRNA expression in circulating EVs from pregnant mice fed a control diet, high fat diet (HFD, a model for GDM) or HFD with lycium barbarum polysaccharide. As compared to control group, 18 miRNAs were found to be differentially expressed in the HFD group (11 upregulated and 7 downregulated). In comparison with HFD group, 16 miRNAs were found to be differentially expressed in the HFD+LBP group (6 downregulated and 10 upregulated). Notably, treatment with LBP was shown to return the expression of 6 miRNAs increased in GDM to normal levels. These changes were seen concurrent with improved glucose tolerance.

Finally, circular RNAs (circRNAs) have also emerged as endogenous non-coding RNA that are enriched in EVs and may also provide insights into molecular signaling. Cao et al.[34] analyzed circRNAs in EVs isolated from umbilical cord blood of GDM and control patients by microarray. From the differentially expressed circRNA they validated differential expression of 12 circDNA (7 increased, 5 decreased, see Table 2). Using bioinformatic tools they suggested that the differentially expressed circRNAs were involved in galactose metabolism, pentose phosphate pathway, glycan biosynthesis, cholesterol metabolism, DNA replication, and RNA transport, which are responsible for metabolic process, growth and development and play role in pathogenesis of GDM.

The above studies show a similar pattern of differential increase in EV miRNA in GDM, though the precise changes reported differ between studies. Although they reported different set of upregulated miRNAs, both Gillet et al.[39] and Nair et al.[41] found that the upregulated miRNAs are involved in mainly phosphatidylinositol 3-kinase-AKT pathway, which plays an important role in insulin and glucose secretion and development of placenta and fetal growth. These studies support the use of placenta derived S-EVs as a screening strategy for early diagnosis of GDM though they differ in type of sample studied and timing of sample taken. Further analyses are required to validate these findings and apply to a larger population and to evaluate their diagnostic or prognostic value in GDM.

## Changes in EV protein content in GDM

While RNA can influence cellular homeostasis in diabetes, high glucose conditions may directly affect protein expression and activity. It has long been appreciated that EVs contain protein that are reflective of their cell of origin.[12]

With advancements in proteomics, numerous studies have conducted large-scale assessment of protein composition of EVs. We and others have shown that changes in EV protein composition can indicate underlying metabolic stress and emerging evidence suggests that EV protein content is altered in GDM.[12,16] Our search identified four reports that undertook studies on EV protein content in GDM (Figure 3).

Jayabalan et al.[36] assessed the production and proteomic content of S-EVs isolated from omental adipose tissue of pregnant women with GDM or normal glucose tolerance (NGT). Omental adipose tissue was obtained and cultured *ex* vivo and S-EVs were isolated from omental AT-conditioned media. The proteomic profile of AT-derived S-EVs (exo-AT) was analyzed using mass spectrometry. The pathway analysis from S-EVs obtained from GDM participants showed enrichment in proteins with the key pathways involved in mitochondrial dysfunction, sirtuin signaling pathway, mechanistic target of rapamycin signaling pathway and oxidative phosphorylation. These alterations may, in turn, regulate fetoplacental metabolism since the expression of genes related to gluconeogenesis and glycolysis in placental cells that were treated with S-EVs from GDM but not NGT adipose tissue.

In another study that was conducted by Jayabalan and colleagues,[43] the authors looked at circulating EVs in plasma samples from women with GDM and those with normal glucose tolerance. The authors observed changes in the expression of circulating S-EVs proteins in plasma at the onset of GDM. A total of 78 proteins showed significantly altered expression in GDM. These proteins were associated with inflammation, metabolism and energy production. The data elucidate the underlying physiological mechanisms that are possibly linked to insulin resistant in GDM.

Kandzija and colleagues[44] investigated the expression of the protein DPP-IV in placental EVs in GDM versus normal glucose tolerance. Using syncytiotrophoblast-derived extracellular vesicles (STB-EVs) from control and GDM, they show that DPPIV-positive STB-EVs released from normal human placenta that were higher in uterine than paired peripheral blood, suggesting placental origin. The authors reported an eightfold increase of DPPIV-bound STB-EVs in the circulation of participants with GDM. Importantly, the authors confirmed that the enzyme retains its functional activity in the EVs. This study is the first to confirm the presence of a biologically active molecule in STB-EVs that may regulate maternal insulin secretion.

Finally, Ramachandrarao et al. reported that urinary S-EVs may be a promising biomarker of diabetes in pregnancy.[45] In this study, the S-EVs protein content of 24 h urine samples from individuals with GDM and pre-
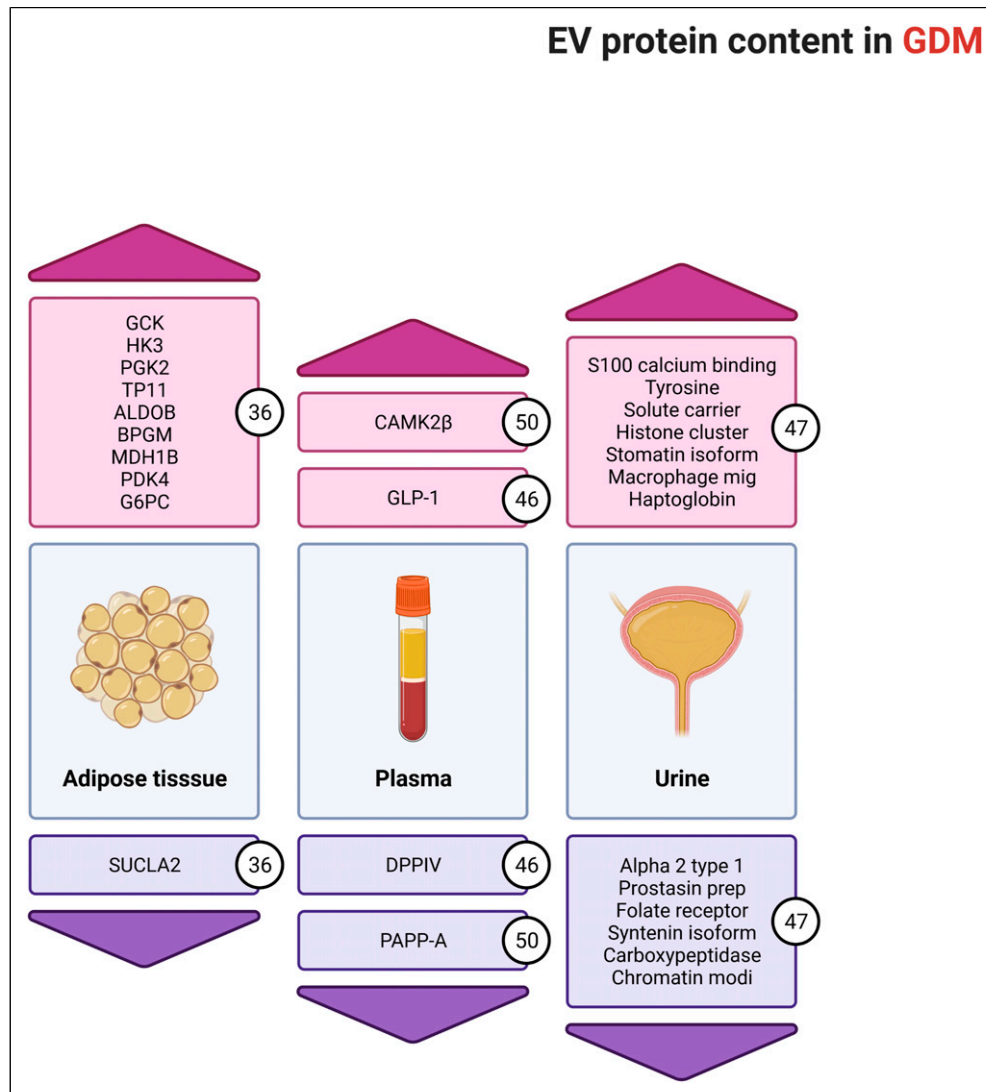
**Figure 3.** EV-associated proteins that are altered in GDM. Shown are proteins that are increased (fuchsia) or down-regulated (violet) in the context of GDM. White circles indicate the reference number for individual studies. Created with BioRender.com.

gestational type 2 diabetes and normal glucose tolerance samples collected at 20 weeks gestation were compared. Using liquid chromatography/mass spectrometry the differences in S-EVs protein load between the groups were detected. The authors identified unique protein signatures in EVs from GDM versus normal glucose tolerance. They identified 646 (CTRL), and 734 (GDM) and 856 (PGD) proteins in S-EVs at 20 weeks of pregnancy. A key observation was that S100 calcium binding protein A9 (damage associated molecular pattern (DAMP) signal) was significantly increased in women with PGD and GDM with similar levels between the two diabetic groups. The peptide counts for S100A9 protein were independently correlated with macrosomia and maternal obesity.

## Relationship between EVs and outcomes in GDM

It is clear that exposure to diabetes *in utero* has short- and long-term effects on the mother and offspring that are reflected in alterations in EV formation, their physical properties, and their molecular composition. A logical extension from this is that changes to EVs may provide insights into the molecular pathogenesis of GDM.

In this regard, Shah et al.[46] examined associations between EV miRNA levels in HUVEC cells and pregnancy outcomes. In particular, they observed a correlation between birth weight and expression of several miRNA species. They observed a negative relationship between birth weight andmiR-130-3b-3p in circulating S-EVs. Similarly they observed negative associations with miR-

126-3p, miR-148-3p and miR-let7a-5p in cultured HU-VECs and miR-130b-3p, miR-29a-3p, and miR-let-7a-5p in placenta. The authors interpreted the above changes as a protective mechanism against macrosomia by attenuation of glucose metabolism and insulin action as these miRNAs target different enzymes in glucose metabolism. There were also significant differences in the expression of miRNA between the infant sex with males having higher expression of miR-126-3p by 35% in S-EVs and higher expression of miR-148-3p and miR-29a-3p in HUVEC. However, it is worth noting that maternal factors like physical activity, diet, weight gain during pregnancy which affect miRNA expression as well as outcomes, were not taken into consideration.

As discussed previously, Ramachandrarao et al.[45] examined the relationships among urinary S-EVs protein concentrations from non-diabetic women and those with GDM or pregestational diabetes. They found that neonate head circumference was similar between GDM and PGD group. However, in PGD group, none of the neonates were macrosomic, whereas in the GDM group, 3 out of 6 were macrosomic. They found that the peptide S100 A9 independently correlated with macrosomia in newborn infants.

Interestingly, Jayabalan et al.[36] in their study of omental adipose tissue S-EVs found a positive correlation between the concentration of S-EVs from adipose tissue explants and infant birthweight Z scores, further strengthening the relationship between S-EVs released from adipose tissue and modulating transplacental nutrient transport leading to fetal overgrowth.

Finally Cao et al.[34] found positive association between S-EV concentration in umbilical cord blood and neonatal birth weight.

## Future Directions

The study of EVs in GDM offers promise for gaining a better understanding of disease pathogenesis and as diagnostic tools. However, work in this area is at its infancy. As detailed above, EV levels, physicochemical properties, and molecular composition may all be altered in GDM. Assessment of such changes may ultimately help identify patients at risk of complications or in monitoring response to interventions as suggested by Xiao et al. It is notable that the focus to date has largely been on placental alterations to date, however maternal changes are also evident, particularly in studies involving urine EVs.

Nevertheless, significant challenges remain before EVs can graduate from research item to clinical diagnostic tool. As evident from the present review, studies examining EVs in GDM have employed distinct sources of EVs (plasma, urine, media from cultured explants…) and distinct approaches to EV isolation and characterization EVs. External validation is lacking for key observations and should be a priority going forward. This is particularly important because many studies have employed a candidate-based approach to assessing EV content rather than a comprehensive unbiased screen.

In addition, while a handful of studies have explored links between EV changes in GDM and clinical variables, these have largely been small cohorts and selected populations. As such, assessing relationships less common outcomes (i.e. preeclampsia, pregnancy loss, NICU admission etc…) may not have been possible. Future studies should focus on larger, more representative study populations to identify strong, reproducible changes that correlate with clinical outcomes.

We also note that studies on EVs in GDM have focused on changes seen during pregnancy. However, it is increasingly apparent that this is a long-term condition that contributes to cardiometabolic disorders in both mother and offspring later in life. Given this, one wonders whether GDM-mediated changes to EV levels persist after pregnancy and return to normal glucose tolerance. It is possible that EVs may show evidence of legacy effects responsible for the long-term effects of GDM.

Finally, one must also consider the fact that EV release is a physiological process that is merely dysregulated under certain conditions. Thus, it is entirely possible that EVs may act in a protective manner under appropriate conditions. Indeed, administration of exogenous EVs (particularly the small "exosome" population) has been shown to be protective in animal models of diabetes.[47–50] To the best of our knowledge, this has yet to be explored in the context of GDM; however, it may represent a novel therapeutic approach.

## ORCID iD

Dylan Burger [ID] https://orcid.org/0000-0003-3951-2911

## References

1. American Diabetes Association. Standards of Medical Care in Diabetes—2014. *Diabetes Care* 2013; 37: S14–S80.
2. Zhu Y and Zhang C. Prevalence of gestational diabetes and risk of progression to type 2 diabetes: a global perspective. *Curr Diab Rep* 2016; 16: 7.

3. Kc K, Shakya S and Zhang H. Gestational diabetes mellitus and macrosomia: a literature review. *Ann Nutr Metab* 2015; 66: 14–20.

4. Schwartz N, Nachum Z and Green MS. The prevalence of gestational diabetes mellitus recurrence-effect of ethnicity and parity: a metaanalysis. *Am J Obstet Gynecol* 2015; 213: 310–317.

5. Bellamy L, Casas J-P, Hingorani AD, et al. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *The Lancet* 2009; 373: 1773–1779.

6. Kramer CK, Campbell S and Retnakaran R. Gestational diabetes and the risk of cardiovascular disease in women: a systematic review and meta-analysis. *Diabetologia* 2019; 62: 905–914.

7. Rawal S, Olsen SF, Grunnet LG, et al. Gestational diabetes mellitus and renal function: a prospective study with 9-to 16-Year follow-up after pregnancy. *Diabetes Care* 2018; 41: 1378, 1384.

8. Wendland EM, Torloni MR, Falavigna M, et al. Gestational diabetes and pregnancy outcomes -a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria. *BMC Pregnancy Childbirth* 2012; 12: 23.

9. Mortier I, Blanc J, Tosello B, et al. Is gestational diabetes an independent risk factor of neonatal severe respiratory distress syndrome after 34 weeks of gestation? A prospective study. *Arch Gynecol Obstet* 2017; 296: 1071–1077.

10. Sheiner E. Gestational diabetes mellitus: long-term consequences for the mother and child grand challenge: how to move on towards secondary prevention? *Front Clin Diabetes Healthc* 2020; 1, https://www.frontiersin.org/article/10.3389/fcdhc.2020.546256 (accessed 9 March 2022).

11. Saeedi M, Cao Y, Fadl H, et al. Increasing prevalence of gestational diabetes mellitus when implementing the IADPSG criteria: a systematic review and meta-analysis. *Diabetes Res Clin Pract* 2021; 172: 108642.

12. Burger D, Schock S, Thompson CS, et al. Microparticles: biomarkers and beyond. *Clin Sci* 2013; 124: 423–441.

13. Witwer KW, Buzas EI, Bemis LT, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *J Extracell Vesicles*; 2, 20360. Epub ahead of print 2013. DOI: 10.3402/jev.v2i0.20360.

14. Sarker S, Scholz-Romero K, Perez A, et al. Placenta-derived exosomes continuously increase in maternal circulation over the first trimester of pregnancy. *J Transl Med* 2014; 12: 204.

15. Livshits MA, Khomyakova E, Evtushenko EGE, et al. Isolation of exosomes by differential centrifugation: theoretical analysis of a commonly used protocol. *Sci Rep* 2015; 5: 17319.

16. Denzer K, Kleijmeer MJ, Heijnen HF, et al. Exosome: from internal vesicle of the multivesicular body to intercellular signaling device. *J Cell Sci* 2000; 113: 3365–3374.

17. van Balkom BW, de Jong OG, Smits M, et al. Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. *Blood* 2013; 121(3997–4006): S1–S15.

18. Akers JC, Gonda D, Kim R, et al. Biogenesis of extracellular vesicles (EV): exosomes, microvesicles, retrovirus-like vesicles, and apoptotic bodies. *J Neurooncol* 2013; 113: 1–11.

19. Théry C, Witwer KW, Aikawa E, et al. 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. *J Extracell Vesicles* 2018; 7: 1535750.

20. Burger D, Turner M, Xiao F, et al. High glucose increases the formation and pro-oxidative activity of endothelial microparticles. *Diabetologia* 2017; 60: 1791–1800.

21. Burger D, Thibodeau J-F, Holterman CE, et al. Urinary podocyte microparticles identify prealbuminuric diabetic glomerular injury. *J Am Soc Nephrol* 2014; 25: 1401–1407.

22. Wang GH, Ma KL, Zhang Y, et al. Caspase 3/ROCK1 pathway mediates high glucose-induced platelet microparticles shedding. *Biochem Biophys Res Commun* 2019; 509: 596–602.

23. Li S, Wei J, Zhang C, et al. Cell-Derived Microparticles in Patients with Type 2 Diabetes Mellitus: a systematic review and meta-analysis. *Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol* 2016; 39: 2439–2450.

24. Sabatier F, Darmon P, Hugel B, et al. Type 1 And Type 2 diabetic patients display different patterns of cellular microparticles. *Diabetes* 2002; 51: 2840–2845.

25. Lytvyn Y, Xiao F, Kennedy CRJ, et al. Assessment of urinary microparticles in normotensive patients with type 1 diabetes. *Diabetologia* 2017; 60: 581–584.

26. Abolbaghaei A, Langlois M-A, Murphy HR, et al. Circulating extracellular vesicles during pregnancy in women with type 1 diabetes: a secondary analysis of the CONCEPTT trial. *Biomark Res* 2021; 9: 67.

27. Jansen F, Wang H, Przybilla D, et al. Vascular endothelial microparticles-incorporated microRNAs are altered in patients with diabetes mellitus. *Cardiovasc Diabetol* 2016; 15: 49.

28. Yáñez-Mó M, Siljander PR-M, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 2015; 4: 27066.

29. Zhang J, Li H, Fan B, et al. Extracellular vesicles in normal pregnancy and pregnancy-related diseases. *J Cell Mol Med* 2020; 24: 4377–4388.

30. Salomon C, Scholz-Romero K, Sarker S, et al. Gestational Diabetes Mellitus Is Associated With Changes in the Concentration and Bioactivity of Placenta-Derived Exosomes in Maternal Circulation Across Gestation. *Diabetes* 2016; 65: 598–609.

31. Arias M, Monteiro LJ, Acuña-Gallardo S, et al. [Extracellular vesicle concentration in maternal plasma as an early

marker of gestational diabetes]. *Rev Med Chil* 2019; 147: 1503–1509.

32. James-Allan LB, Rosario FJ, Barner K, et al. Regulation of glucose homeostasis by small extracellular vesicles in normal pregnancy and in gestational diabetes. *FASEB J Off Publ Fed Am Soc Exp Biol* 2020; 34: 5724–5739.

33. Franzago M, Lanuti P, Fraticelli F, et al. Biological insight into the extracellular vesicles in women with and without gestational diabetes. *J Endocrinol Invest* 2021; 44: 49–61.

34. Cao M, Zhang L, Lin Y, et al. Circular RNA expression profiles in umbilical cord blood exosomes from normal and gestational diabetes mellitus patients. *Biosci Rep* 2020; 40: BSR20201946.

35. Monteiro LJ, Varas-Godoy M, Monckeberg M, et al. Oral extracellular vesicles in early pregnancy can identify patients at risk of developing gestational diabetes mellitus. *PloS One* 2019; 14: e0218616.

36. Jayabalan N, Lai A, Ormazabal V, et al. Adipose tissue exosomal proteomic profile reveals a role on placenta glucose metabolism in gestational diabetes mellitus. *J Clin Endocrinol Metab* 2019; 104: 1735–1752.

37. Sáez T, de Vos P, Kuipers J, et al. Fetoplacental endothelial exosomes modulate high d-glucose-induced endothelial dysfunction. *Placenta* 2018; 66: 26–35.

38. van Balkom BWM, Eisele AS, Pegtel DM, et al. Quantitative and qualitative analysis of small RNAs in human endothelial cells and exosomes provides insights into localized RNA processing, degradation and sorting. *J Extracell Vesicles* 2015; 4: 26760.

39. Gillet V, Ouellet A, Stepanov Y, et al. miRNA profiles in extracellular vesicles from serum early in pregnancies complicated by gestational diabetes mellitus. *J Clin Endocrinol Metab* 2019; 104: 5157–5169.

40. Herrera-Van Oostdam AS, Toro-Ortíz JC, López JA, et al. Placental exosomes isolated from urine of patients with gestational diabetes exhibit a differential profile expression of microRNAs across gestation. *Int J Mol Med* 2020; 46: 546–560.

41. Nair S, Jayabalan N, Guanzon D, et al. Human placental exosomes in gestational diabetes mellitus carry a specific set of miRNAs associated with skeletal muscle insulin sensitivity. *Clin Sci Lond Engl* 2018; 132: 2451–2467.

42. Xiao Y, Chen W, Chen R, et al. Exosomal microrna expression profiling analysis of the effects of lycium barbarum polysaccharide on gestational diabetes mellitus mice. *Evid-Based Complement Altern Med ECAM* 2020; 2020: 2953502.

43. Jayabalan N, Lai A, Nair S, et al. Quantitative Proteomics by SWATH-MS suggest an association between circulating exosomes and maternal metabolic changes in gestational diabetes mellitus. *Proteomics* 2019; 19: e1800164.

44. Kandzija N, Zhang W, Motta-Mejia C, et al. Placental extracellular vesicles express active dipeptidyl peptidase IV; levels are increased in gestational diabetes mellitus. *J Extracell Vesicles* 2019; 8: 1617000.

45. Ramachandrarao SP, Hamlin AA, Awdishu L, et al. Proteomic analyses of urine exosomes reveal new biomarkers of diabetes in pregnancy. *Madridge J Diabetes* 2016; 1: 11–22.

46. Shah KB, Chernausek SD, Teague AM, et al. Maternal diabetes alters microRNA expression in fetal exosomes, human umbilical vein endothelial cells and placenta. *Pediatr Res* 2021; 89: 1157–1163.

47. He Q, Wang L, Zhao R, et al. Mesenchymal stem cell-derived exosomes exert ameliorative effects in type 2 diabetes by improving hepatic glucose and lipid metabolism via enhancing autophagy. *Stem Cell Res Ther* 2020; 11: 223.

48. Sun Y, Shi H, Yin S, et al. Human mesenchymal stem cell derived exosomes alleviate Type 2 Diabetes mellitus by reversing peripheral insulin resistance and relieving β-Cell Destruction. *ACS Nano* 2018; 12: 7613–7628.

49. Bai S, Yin Q, Dong T, et al. Endothelial progenitor cell-derived exosomes ameliorate endothelial dysfunction in a mouse model of diabetes. *Biomed Pharmacother Biomedecine Pharmacother* 2020; 131: 110756.

50. Burger D, Vinas JL, Akbari S, et al. Human endothelial colony-forming cells protect against acute kidney injury: role of exosomes. *Am J Pathol* 2015; 185: 2309–2323.

## Appendix S1: Search Strategy

Database: Embase Classic+Embase <1947 to 2020 December 07>, Ovid MEDLINE(R) ALL <1946 to 04 December 2020>

Search Strategy:

1. extracellular vesicles/or cell-derived microparticles/or exosomes/ (47,352)
2. (extracellular vesicle* or Ectosome* or Exosome* or microparticle* or microvesicle*or sevs).tw,kf. (89,467)
3. 1 or 2 (96,357)
4. exp Diabetes, Gestational/ (52,045)
5. exp Pregnancy in Diabetics/ (51,985)
6. ((pregnan* or gestat* or maternal) adj5 diabet*).tw,kf. (66,917)
7. ((Fetus or foetus or feti or feotuses or Fetal or Foetal or placenta*) adj5 diabet*).tw,kf. (7892)
8. (Pregnancy/or placenta/or fetus/) and exp Diabetes Mellitus/ (57,918)
9. 4 or 5 or 6 or 7 or 8 (100,456)
10. 3 and 9 (180)
11. 10 use medall (56) Medline
12. exosome/ (30,612)
13. membrane microparticle/(5571)

14. (microparticle* or ectosome* or exosome* or microvesicle* or extracellular vesicle* or sevs).tw. (94,027)
15. 12 or 13 or 14 (98,155)
16. pregnancy diabetes mellitus/or maternal diabetes mellitus/ (39,061)
17. ((pregnan* or gestat* or maternal) adj5 diabet*).tw. (66,205)
18. ((Fetus or foetus or feti or feotuses or Fetal or Foetal or placenta*) adj5 diabet*).tw. (7846)
19. (pregnancy/or placenta*.mp. or fetus/) and (diabetes mellitus/or non insulin dependent diabetes mellitus/) (28,861)
20. 16 or 17 or 18 or 19 (92,870)
21. 15 and 20 (178)
22. 21 use emczd (130) Embase
23. 11 or 22 (186)
24. remove duplicates from 23 (138)
25. 24 use medall (56) Medline
26. 24 use emczd (82) Embase