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Publication Date
2021-09-01

DOI
10.1016/j.placenta.2021.02.012

Peer reviewed

HHS Public Access

Author manuscript

Placenta. Author manuscript; available in PMC 2022 September 15.

Published in final edited form as:

Placenta. 2021 September 15; 113: 15–22. doi:10.1016/j.placenta.2021.02.012.

Extracellular vesicles and their role in gestational diabetes mellitus

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Abstract

Gestational diabetes mellitus (GDM) is a complex disorder that is defined by glucose intolerance with onset during pregnancy. The incidence of GDM is increasing worldwide. Pregnancies complicated with GDM have higher rates of maternal and fetal morbidity with short- and long-term consequences, including increased rates of cardiovascular disease and type II diabetes for both the mother and offspring. The pathophysiology of GDM still remains unclear and there has been interest in the role of small extracellular vesicles (sEVs) in the maternal metabolic adaptations that occur in pregnancy and GDM. Small EVs are nanosized particles that contain bioactive content, including miRNAs and proteins, which are released by cells to provide cell-to-cell communication. Pregnancy induces an increase in total and placental-secreted sEVs across gestation, with a further increase in sEV number and changes in the protein and miRNA composition of these sEVs in GDM. Research has suggested that these sEVs have an impact on maternal adaptations during pregnancy, including targeting the pancreas, skeletal muscle and adipose tissue. Consequently, this review will focus on the differences in total and placental sEVs in GDM compared to normal pregnancy, the role of sEVs in the pathophysiology of GDM and their clinical application as potential GDM biomarkers.

Keywords

Pregnancy; Placenta; Extracellular vesicles; Exosomes; Microvesicles; Gestational diabetes mellitus; miRNAs; Proteins

1. Introduction

The extracellular vesicle (EV) field is novel, innovative and fast-moving and there have been a number of reviews detailing the role of EVs in reproduction [1,2] and complications of pregnancy [3–5], as well as focusing on placental EVs [6,7] and placental-derived miRNAs packaged in EVs [8]. However, there is a shortage of reviews that focus on EVs in gestational diabetes mellitus (GDM). The review published by Iljas et al. [9] details the original studies in this field followed by reviews by Nair et al. [10,11] and Floriano et al. [12] detailing the roles of EVs in maternal insulin production and metabolism during GDM and the translational aspect of EVs as therapeutic agents for GDM, respectively. Our present

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review expands upon the evidence that EVs play a key role in the pathogenesis of GDM, focusing mainly on the miRNA and protein content of EVs, maternal-fetal communication and the clinical applications of EVs in GDM.

2. Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) is defined as glucose intolerance with onset or first recognition during pregnancy. It affects ~2-14% of pregnancies worldwide and the prevalence is increasing, with the number of cases up by 56% between 2000 and 2010 in the USA [13–15]. This increase is believed to be linked to the rising incidence of obesity. There is also a racial and ethnic disparity in GDM cases, with non-white minorities having a much higher prevalence of developing GDM [15–17]. Other risk factors besides obesity, are advanced maternal age (AMA), a family history of diabetes and GDM in a prior pregnancy [18]. Pregnancy is characterized by the development of relative maternal insulin resistance that begins in mid-to late-gestation to allow for selective transport of maternal nutrients across the placenta rather than into other maternal tissues, to support exponential growth and development of the fetus through gestation. In GDM, there is pronounced insulin resistance leading to episodic fasting and postprandial maternal and fetal hyperglycemia. Consequently, this can lead to pregnancy-induced hypertension, premature rupture of amniotic membranes, cesarean section and preterm delivery [19]. In the long-term, women with GDM have an increased risk of developing type-2 diabetes and cardiovascular disease subsequent to pregnancy [20–22]. Additionally, mild forms of GDM are associated with fetal overgrowth, while moderate to severe GDM results in fetal growth restriction, both conditions elevating perinatal morbidity and mortality and increasing the risk of adiposity/obesity [23,24], cardiovascular disease [25] and type-2 diabetes [26] later in life for the offspring. In female offspring, an increased chance of developing GDM themselves in pregnancy leads to a self-perpetuating transgenerational cycle of pathology.

Despite the increasing prevalence of GDM, the pathophysiology is not fully understood, including the inter-connectivity and molecular communication between the mother and fetus. Consequently, it is crucial to understand this in more detail to reduce the overall incidence and the morbidity associated with GDM, ultimately, improving diagnostic testing modalities and advancing innovative treatment options. In doing so, this will improve both short- and long-term health for women and infants born to GDM mothers.

3. Extracellular vesicles

Extracellular vesicles are nanosized particles that contain bioactive content, including proteins, lipids and nucleic acids, enclosed by a lipid bilayer. They are released by most cells and are found in a diverse range of bodily fluids, including blood, urine, breast milk, amniotic fluid, cerebrospinal fluid, semen, ascites fluid, bile and saliva. The classification of EVs is an ongoing matter of debate [27], however most commonly in the literature EVs are classified into three main groups–exosomes, microvesicles and apoptotic bodies. Exosomes, which range from ~50 to 150 nm in diameter, are of endosomal origin and are released as a consequence of multivesicular bodies (MVB) fusing with the plasma membrane. EVs that are formed and released from the plasma membrane are generally termed microvesicles,

also referred to as ectosomes, microparticles or shedding vesicles; these range in size from ~100 to 1000 nm in diameter. Apoptotic bodies are produced by cellular plasma membrane blebbing that occurs with apoptosis and are typically >1000 nm.

The International Society of Extracellular Vesicles (ISEV) recommends the use of EVs as the generic term for particles released by cells that have a lipid bilayer and do not replicate [27]. It is known that cells release a heterogenous population of EVs; ranging in size, density and content. However, EV isolation methods and characterization lack the specificity to currently delineate specific subtypes of EVs, which often overlap in size and density. With a lack of specific EV subtype markers [28] it has been suggested to use nomenclature that pertains to physical characteristics, such as size, biochemical composition, or the cell of origin [27]. Consequently, in this review we will use small EVs (sEVs) as a generic term for both exosomes and microvesicles.

4. Extracellular vesicles in pregnancy

During pregnancy the syncytiotrophoblast layer of the placenta secretes sEVs into the maternal circulation from as early as 6 weeks of gestation [29]. Placental EVs (pEVs) are characterized by the presence of the syncytiotrophoblast marker placental alkaline phosphatase (PLAP), found on the surface of sEVs released from syncytiotrophoblast cells [30]. Salomon et al. have demonstrated that the circulating concentration of both total and pEVs in maternal plasma significantly increase during pregnancy, and that the concentrations of both total and pEVs are positively correlated with advancing gestation [31].

Although sEVs predominantly traffic from the placenta to the maternal circulation, there is evidence that maternal sEVs and pEVs transport to the fetal compartment during pregnancy [32,33] and that fetal sEVs are able to traffic to maternal compartments [34]. Sheller-Miller et al. have demonstrated that maternal sEVs can travel to fetal tissues leading to functional changes during pregnancy therefore, indicating that sEVs can assist in paracrine feto-maternal communication during pregnancy [33]. The discovery of fetal sEVs, as well as pEVs, in the maternal circulation could lead to a noninvasive tool for identifying changes in fetal and placental health. Such modalities can aid in developing earlier diagnostic tests and provide possibilities for early interventions targeting prevention of pregnancy associated complications, such as GDM.

The release and content of sEVs is dependent on the microenvironment; during gestation the release of pEVs is regulated by certain metabolic factors, such as hypoxia and glucose [35,36]. EVs released from the placenta contain a heterogenous array of proteins [37] and miRNAs [38–44]. It is thought that pEVs modulate feto-maternal communication essential for adaptations that are necessary during pregnancy. Conversely, it is known that sEVs contribute to various pathologies observed in pregnancy, such as in GDM [12]. Consequently, sEVs provide a mode of maternal-fetal communication and vice versa during gestation, by transferring their cargo, which in turn reflects the functional state of the cells of origin, to recipient cells causing phenotypic changes observed in both healthy and abnormal pregnancies.

5. Extracellular vesicles in GDM

Pregnant women who go on to develop GDM display increased concentration of both total and pEVs compared to their normoglycemic counterparts [45]. This GDM associated increase in circulating sEVs continues throughout pregnancy, with a ~2-fold increase in the number of circulating pEVs at term in GDM compared to healthy pregnancies [46]. GDM is associated with maternal hyperglycemia which in turn can stimulate the release and bioactivity of pEVs [36,46].

Normal pregnancy is associated with inflammation, however this is further exacerbated in GDM demonstrated by an increase in inflammatory markers [47]. Increased inflammation is linked to a higher susceptibility of developing GDM and there is evidence that elevated expression of pro-inflammatory cytokines may be linked to insulin resistance as observed in GDM. Placental EVs have been shown to be bioactive and contribute to the maternal pro-inflammatory state observed in GDM by stimulating the release of pro-inflammatory cytokines from human umbilical vein endothelial cells [45]. Activation of endothelial cells leads to an increased release of sEVs from these cells, which may mediate the fetoplacental endothelial dysfunction observed in GDM. The release of vascular endothelial sEVs is beyond the scope of this review but is described in more detail by Saez et al. [48].

Human pregnancy is characterized by changes in maternal metabolism to ensure preferential allocation of nutrients to the fetus to fuel necessary growth and development. This process is defined by changes in production, secretion and action of insulin on peripheral tissue glucose uptake and utilization, leading to insulin resistance. However, in pregnancies complicated with GDM there is insufficient maternal insulin secretion from pancreatic β-islet cells causing marked insulin resistance as compared to normoglycemic pregnancies. There is evidence that sEVs are involved in the metabolic adaptation that occurs in normal pregnancy and in the metabolic dysfunction observed in GDM. This was demonstrated in a study where total sEVs isolated from healthy pregnant women were infused into healthy mice inducing pancreatic β-islet cell glucose stimulated insulin secretion resulting in increased fasting insulin concentrations and skeletal muscle insulin resistance [49]. In contrast, infusion of sEVs isolated from women with GDM in to healthy mice induced glucose intolerance, failed to promote β-islet cellular glucose stimulated insulin secretion and inhibited skeletal muscle insulin signaling [49]. This suggests that sEVs, at least in part secreted by the placenta, contribute to the pathophysiology of GDM, including dysregulated endogenous insulin secretion and insulin resistance in peripheral tissues. Additionally, pEVs have been shown to modulate skeletal muscle cell migration and insulin sensitivity in normal pregnancy, whereas in pEVs from women with GDM skeletal muscle cell migration was reduced and insulin signaling was attenuated in skeletal muscle cells [38]. These results suggest that pEVs have a significant effect on insulin signaling in skeletal muscle and the altered composition of pEVs in GDM have an effect on the maternal metabolism observed in GDM pregnancies.

During pregnancy, there is expansion of adipose tissue to support nutrient transport to the fetus. However, GDM is associated with reduced adipocyte differentiation and adipocyte hypertrophy, as well as downregulation of key insulin signaling regulators and adipogenic

transcription factors, which leads to insulin resistance in this tissue. Jayabalan et al. discovered that adipose tissue from women with GDM secrete an increased number of sEVs compared to women with normal glucose tolerance during pregnancy. This increased number of sEVs correlates positively with birthweight of the offspring, suggesting a possible additional role in fetal overgrowth commonly observed in GDM [50]. Conversely, Franzago et al. observed a lower concentration of adipocyte sEVs in maternal circulation in women with GDM compared to normal pregnancies, however this group discovered a correlation between adipocyte sEVs and total cholesterol levels in women with GDM [51]. Despite the differences in these observations differential expression of sEV protein cargo is associated with downregulation of the sirtuin signaling pathway and upregulation of oxidative phosphorylation pathways and mTOR signaling in adipose tissue from women with GDM suggesting that adipose tissue derived sEVs may contribute to the pathogenesis of GDM [50]. Additionally, studies show that adipose tissue derived sEVs may mediate metabolic communication between adipose tissue and the placenta and by doing so enhance glucose uptake and metabolism in the placenta of women with GDM [50].

In addition to sEVs having an effect on maternal metabolism in GDM, studies have demonstrated that maternal sEVs are able to traffic across the placental barrier from the maternal circulation to the fetus, leading to functional fetal changes [32,33]. In GDM, maternal sEVs cross the maternal-fetal barrier and are endocytosed by fetal tissues [52]. Differential expression of miRNAs in maternal sEVs is thought to contribute to the congenital cardiac developmental deficits observed in the offspring born to GDM mothers [52]. The discovery of sEVs with aberrant mRNA expression, associated with glucose metabolism and growth signaling pathways, in umbilical cord blood of GDM pregnancies suggests that these sEVs may target the fetus and have a role in fetal growth and metabolism [53]. Although there is evidence of maternal-fetal communication in pregnancy, currently there are few studies that have investigated how maternal sEVs and their cargo target the fetus in GDM. However, studies have demonstrated that children from GDM complicated pregnancies have altered expression of miRNAs associated with diabetes, cardiovascular and cerebrovascular disease with associated links to cardiovascular disease [54]. Although this study did not investigate sEV encapsulated miRNAs, these results suggest that GDM leads to long-term changes in the miRNA profile of children born to GDM mothers, therefore impacting the long-term health of these children. Consequently, future investigations are required to elucidate the effect that maternal sEVs in GDM have on the fetus, including the short-and long-term complications observed in infants born to GDM mothers.

5.1. sEV miRNAs in GDM

miRNAs are small non-coding single-stranded RNAs (21-24 nucleotides) that regulate gene expression at the post-transcriptional level through sequence-specific base-pairing with the 3'-UTRs of target mRNAs. The placenta expresses a unique miRNA signature, including three clusters, including C19 miRNA cluster (C19MC), C14MC and miR-371-3 cluster [55,56]. Placentally expressed miRNAs have a predominant role in placental development and function, including trophoblast cell proliferation, apoptosis, migration, invasion and angiogenesis [57]. Studies have demonstrated that placenta derived miRNAs are released from syncytiotrophoblasts into the maternal circulation in pEVs [58,59]. At term, the

C19MC is highly expressed in sEVs released from trophoblast cells, whereas in early gestation there is increased expression of C14MC released from sEVs, suggesting that these sEV-encapsulated miRNAs have differing roles throughout gestation [55,60].

There is evidence that miRNAs released via sEVs are involved in GDM pathogenesis as demonstrated by the differential expression of miRNAs during gestation in the maternal circulation of women with GDM compared to healthy normoglycemic pregnancies. Additionally, upregulation of Drosha and Dicer enzymes, key components of the mature miRNA synthesis machinery, found in the circulation of women with GDM suggests dysregulation of miRNAs [61]. GDM modifies the miRNA profile contained within sEVs released from the placenta into the maternal circulation (Table 1). Dysregulation of many miRNAs early in gestation, prior to the diagnosis of GDM at 24-28 weeks, suggests that they are involved in the pathogenesis rather than being a consequence of the subsequent development of metabolic changes characteristic of GDM. Nair et al. identified a set of differentially expressed miRNAs in pEVs from women with GDM [38]. The authors found evidence that in GDM pEVs release miRNAs that interact with genes associated with insulin signaling within skeletal muscle cells thereby potentially contributing to insulin resistance observed in GDM. Other studies have also identified miRNAs in the maternal circulation that are deregulated in women with GDM, these miRNAs are associated with pathways that are involved in GDM including, insulin signaling, type 2 diabetes signaling, mTOR signaling, MAPK signaling, TGFβ signaling and AMPK signaling [39,44].

Although the concentration of both total sEVs and pEVs is higher in GDM in comparison to normal pregnancy, the ratio of pEVs to total sEVs is lower in GDM suggesting increased release of sEVs from non-placental tissue sources. Studies demonstrate that some of the sEV-encapsulatated miRNAs found in the maternal circulation arise from the placenta [38]. However, many of these circulating miRNAs are not part of the placental-specific miRNA clusters which suggests that either they are from other maternal tissue(s) or secreted from placental sEVs while not being placenta-specific. However, without further analysis it is unclear as to the source of these circulating miRNAs and future work in this field is required to delineate the cellular source of sEVs and their miRNA cargo in normal pregnancy and GDM.

sEVs, including pEVs, can be detected in the urine of pregnant women and the miRNA expression in these sEVs differs across gestation, potentially to facilitate the differing needs of the growing and developing fetus with advancing pregnancy [62]. sEVs isolated from the urine of women with GDM have a distinct miRNA expression as compared to miRNAs encapsulated in sEVs from normoglycemic pregnancies. miR-517-5p and miR-518-3p, part of the C19MC, and miR-16-5p were found to be upregulated in urine collected at the second trimester in women who subsequently developed GDM versus those who did not, bearing normal pregnancies. Whereas, in the third trimester of pregnancy, downregulation of miR-222-3p, miR-516-5p, miR-517-3p and miR-518-5p was noted in women with GDM, these differences may have resulted due to the lack of adjustments in the study for interventions (e. g. metformin, insulin) targeting GDM rather than GDM per se.

5.2. sEV proteins in GDM

It is known that sEVs can also contain proteins as part of their cargo, and these proteins contribute towards the cell-to-cell communication of sEVs. It has been shown that the proteomic content of circulating sEVs from women with GDM is significantly different from that of normal pregnancies (Table 2), influencing the maternal pathophysiology [37]. Using quantitative proteomics Jayabalan et al. found differential expression of proteins in sEVs derived from women with GDM versus normoglycemic women. Further validation provided evidence of downregulation of Pappalysin-1 (PAPP-A) and upregulation of calcium/calmodulin dependent protein kinase II beta (CAMK2β) in GDM sEVs [37]. PAPP-A, a glycoprotein, is synthesized by cytotrophoblast cells with lower levels associated with a reduced risk of developing GDM as PAPP-A concentrations in early pregnancy may be linked to insulin sensitivity [76]. CAMK2 is a serine/threonine specific protein kinase and targets insulin receptor signaling and thereby systemic insulin sensitivity, however its specific role in GDM is yet to be determined. Kandzija et al. discovered that the glycoprotein dipeptidyl peptidase IV (DPPIV) in its enzymatically active form is present in sEVs released from the placenta [75]. DPPIV regulates insulin secretion which is important for glucose metabolism. An eightfold increase in circulating DPPIV-positive pEVs was observed in GDM, suggestive of a role in mediating maternal insulin sensitivity.

6. Extracellular vesicles - clinical applications in GDM

It is becoming more widely accepted that improving screening and diagnosis for GDM and performing this earlier in gestation can lead to improved health for the mother and offspring during childhood and in adult life. Currently, GDM is diagnosed by an oral glucose tolerance test between 24 and 28 weeks of gestation [81] and the symptoms are subsequently treated by lifestyle modifications, insulin or insulin-sensitizer drugs. It is known that GDM management can have a positive impact on short-term symptoms but not the longer-term complications associated with GDM [82,83]. This may be because by this point in gestation the fetus has already been exposed to hyperglycemic conditions and placentation has occurred. Therefore, there is little opportunity to reverse any placental changes that may be influencing the maternal metabolic adaptations of GDM. Additionally, most women are not aware of their glycemic status prior to testing that is undertaken during pregnancy. Consequently, if screening and diagnosis could occur earlier in gestation, for example in the first or early second trimester, interventions could be utilized earlier in pregnancy, reversing disease onset and subsequently improving the outcomes and health of both the mother and offspring.

Subsequently, there has been an increase in research investigating prospective biomarkers for improved GDM diagnostics that can be performed earlier in gestation. Small EVs are promising biomarker candidates as 1) the placenta releases sEVs in to the maternal circulation from as early as 6 weeks of gestation; 2) the concentration of both total and placental sEVs is increased in GDM and 3) circulating sEVs in women with GDM contain a unique cargo, including miRNAs (Table 1) and proteins (Table 2) [31,45]. Many of the studies to date have focused on miRNAs and there have been various studies investigating miRNAs in maternal plasma in early gestation and in urine as potential predictors of

GDM. The benefits of using urine in a clinical application are that it is available in large quantities and collection is easy and non-invasive. Consequently, more studies are required to investigate the use of miRNAs encapsulated in EVs from urine as a potential early detection biomarker for GDM.

There have also been alternate biological fluid sEV biomarkers, for instance Monteiro et al. who discovered a higher concentration of oral sEVs in gingival crevicular fluid (GCV) in early gestation in women who went on to develop GDM compared to normoglycemic women [84]. This suggests that the hyperglycemic and pro-inflammatory state of GDM stimulates the release of sEVs into oral fluids. Therefore, these oral GCV sEVs could be a potential first trimester biomarker for GDM in pre-symptomatic women.

The greatest limitations in many of these studies are 1) relatively small study sample size and 2) differing methods used in isolating and characterizing the sEVs described, which often leads to inter-study variation. Consequently, for a clinically applicable sEV-derived GDM biomarker there needs to be more transparency and standardization in the sEV isolation methodology and implementation of larger-scale investigations with adequate power to negate the null hypothesis.

7. Conclusion

In summary, there is growing evidence that circulating sEVs have an important role in pregnancy and the increase in both total and placental sEVs and the change in their cargo in GDM leads to changes in maternal physiology, targeting maternal skeletal muscle, adipose tissue and the pancreas, and therefore contributing to the GDM pathophysiology (Fig. 1). Subsequently, differentially expressed proteins and miRNAs enclosed in sEVs provide possibilities of clinical applications for sEVs by developing improved diagnostic biomarkers for GDM.

Acknowledgements

Fig. 1 was created with BioRender.com.

Funding

Laura James-Allan and Sherin Devaskar are supported by grants from the National Institutes of Health (HD-089714 and HD00015).

Abbreviations

GDM	Gestational diabetes mellitus
sEV	Small extracellular vesicles
pEV	Placental extracellular vesicles
PLAP	Placental alkaline phosphates
miRNA	Micro ribonuclease
mRNA	Messenger ribonuclease

C19MC	Chromosome 19 miRNA cluster
C14MC	Chromosome 14 miRNA cluster

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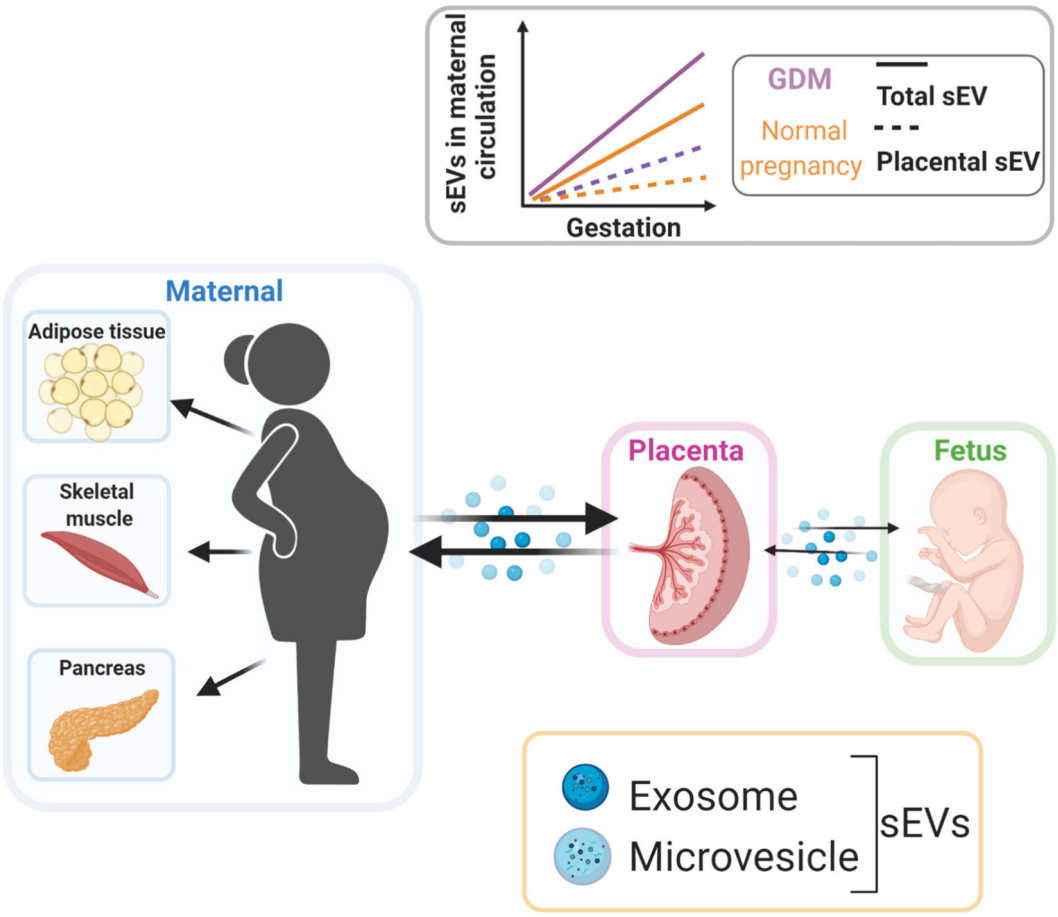

Fig. 1.

Schematic diagram of the release and actions of small extracellular vesicles (sEVs) in gestational diabetes mellitus (GDM) during pregnancy. In GDM there is an increase in the concentration of total and placental sEVs in the maternal circulation across gestation. The release of sEVs during pregnancy leads to communication between the maternal and fetal systems via the placenta. In GDM, sEVs have been shown to play a role in the pathogenesis of GDM by targeting adipose tissue, skeletal muscle and the pancreas in the maternal system.

Placenta. Author manuscript; available in PMC 2022 September 15.

Table 1

Dysregulated miRNAs in sEVs isolated from women with gestational diabetes mellitus (GDM).

miRNA	Source	Trimester of pregnancy	sEV Isolation Method	Differential expression in GDM	Placental specific (C19MC, C14MC)		Target		References
					Y/N	Cluster	Gene/pathway	Organ	
miR-16-5p	Urine	Second trimester	Urine Exosome Purification and RNA Isolation Midi kit	Upregulated	N		PI3K/Akt signaling Wnt/insulin signaling mTOR signaling pathways	Adipose tissue Liver	[62]
miR-29a-3p	Serum	First/early second trimester	Differential centrifugation	Upregulated	N	–	Insulin signaling PI3K-Akt signaling PPAR signaling	Liver Pancreas Skeletal muscle	[63–65]
miR-29b-3p	Serum	First/early second trimester	Differential centrifugation	Upregulated	N	–	Glucose homeostasis Insulin signaling PI3K-Akt signaling	Liver pancreas Skeletal muscle	[64,66]
miR-99b-5p	Plasma	Third trimester	Ultracentrifugation and discontinuous iodixanol gradient centrifugation	Upregulated	N	–	Unknown	Skeletal muscle Placenta	[38]
miR-122-5p	Serum	First/early second trimester	Differential centrifugation	Upregulated	N	–	AMPK signaling pathway Key regulator in insulin resistance Lipid metabolism	Liver Pancreas	[66,67]
miR-125a-3p	Plasma	Third trimester	Ultracentrifugation and discontinuous iodixanol gradient centrifugation	Upregulated	N	–	Insulin signaling pathway	Adipose tissue Skeletal muscle	[63,68]
miR-132-3p	Serum	First/early second trimester	Differential centrifugation	Upregulated	N	–	Insulin receptor signaling pathway AMPK signaling pathway Inhibit GLUT2	Pancreas	[44]
miR-136-5p	Serum	First/early second trimester	Differential centrifugation	Upregulated	Y	C14MC	E2F1 gene leading to impact on cell proliferation	Placenta	[69] [44]
miR-182-3p	Serum	First/early second trimester	Differential centrifugation	Upregulated	N	–	Insulin receptor Inhibit GLUT2 Glucose metabolism Negative regulator of adipogenesis	Pancreas Liver Adipose tissue	[68,69,70]
miR-200a-3p	Plasma	Third trimester	Ultracentrifugation and discontinuous iodixanol gradient centrifugation	Upregulated	N	–	Insulin signaling pathway	Skeletal muscle Placenta Pancreas	[71,72]
miR-210-3p	Serum	First/early second trimester	Differential centrifugation	Upregulated	N	–	Inhibits insulin binding to the insulin receptor Inhibits AMPK Hypoxia	Placenta	[44]

miRNA	Source	Trimester of pregnancy	sEV Isolation Method	Differential expression in GDM	Placental specific (C19MC, C14MC)		Target		References
					Y/N	Cluster	Gene/pathway	Organ	
miR-222-3p	Urine	Third trimester	Urine Exosome Purification and RNA Isolation Midi kit	Downregulated	N		Targets GLUT4 expression	Skeletal muscle	[70,73]
miR-342-3p	Serum	First/early second trimester	Differential centrifugation	Upregulated	Y	C14MC	Inhibits AMPK signaling Inhibits GLUT2 MAPK signaling	Pancreas Placenta	[44]
miR-520h	Serum	First/early second trimester	Differential centrifugation	Upregulated	Y	C19MC	Insulin signaling pathway Glucose metabolism	Pancreas Placenta	[72,74]
miR-516-5p	Urine	Third trimester	Urine Exosome Purification and RNA Isolation Midi kit	Downregulated	Y	C19MC	Unknown	Placenta Adipose tissue	[62]
miR-517-5p	Urine	Second trimester Third trimester	Urine Exosome Purification and RNA Isolation Midi kit	Upregulated Downregulated	Y	C19MC	Unknown	Placenta Adipose tissue	[62]
miR-518-3p	Urine	Second trimester Third trimester	Urine Exosome Purification and RNA Isolation Midi kit	Upregulated Downregulated	Y	C19MC	Regulated PPARα expression	Placenta Adipose tissue	[73,75]
miR-1323	Serum	First/early second trimester	Differential centrifugation	Upregulated	Y	C19MC	Targets genes in the AMPK pathway, predicted to inhibit β-oxidation and glucose transport	Unknown	[44]

Table 2

Dysregulated proteins in sEVs isolated from women with gestational diabetes mellitus (GDM).

Protein name	Protein type	EV Source	Gestation	Expression in GDM	Tissue	Role	References
Pappalysin (PAPP-A)	Glycoprotein	Maternal plasma	22–28 weeks	Downregulated	Villous and extravillous cytotrophoblasts	• Possible role in glucose and insulin sensitivity in pregnancy	[74,66]
Calcium/calmodulin dependent protein kinase II beta (CAMK2β)	Serine/threonine specific protein kinase	Maternal plasma	22–28 weeks	Upregulated	Skeletal muscle	• Role in insulin signaling in skeletal muscle in GDM	[63,77]
Dipeptidyl peptidase IV (DPPIV)	Glycoprotein	Placenta	39–40 weeks	Upregulated	Syncytiotrophoblasts	• Regulates insulin secretion • Role in maternal insulin sensitivity	[75]
Perilipin 4 (PLIN4)	Lipid droplet binding protein	Maternal plasma	22–28 weeks	Upregulated	Adipose tissue Skeletal muscle	• Regulates fat storage • Potential role in insulin resistance and obesity	[78,79,68]
Fatty acid binding protein 3 (FABP3)	Binding protein	Maternal plasma	22–28 weeks	Downregulated	Adipose tissue Placenta	• Increased expression in the	[37]
Hexokinase-3 (HK3)	Enzyme	Maternal plasma	22–28 weeks	Downregulated	Ubiquitous	• Glucose metabolism	[37]
Cortactin binding protein 2 (CTTNBP2)	Binding protein	Maternal plasma	22–28 weeks	Upregulated	Skeletal muscle	• Involved in insulin induced GLUT4 translocation and glucose uptake in skeletal muscle	[80,69]