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Placental extracellular vesicles in maternal-fetal communication during pregnancy

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Abstract

For several years, a growing number of studies have highlighted the pivotal role of placental extracellular vesicles (EVs) throughout pregnancy. These membrane nanovesicles, heterogeneous in nature, composition and origin, are secreted by several trophoblastic cell types and are found in both the maternal and fetal compartments. They can be uptaken by recipient cells and drive a wide variety of physiological and pathological processes. In this review, we provide an overview of the different described roles of placental EVs in various aspects of normal pregnancy, from placenta establishment to maternal immune tolerance towards the fetus and protection against viral infections. In a second part, we present selected examples of pathological pregnancies in which placental EVs are involved, such as gestational diabetes mellitus, pre-eclampsia, and congenital infections. Since the abundance and/or composition of placental EVs is deregulated in maternal serum during pathological pregnancies, this makes them interesting candidates as non-invasive biomarkers for gestational diseases and opens a wide field of translational perspectives.

Introduction

Extracellular vesicles (EVs) are membrane-bound vesicles released from virtually all cells into the extracellular space [1]. EVs comprise vesicles that originate from the endosomal network (exosomes) or from the outward budding of the plasma membrane (microparticles, oncosomes, or microvesicles, collectively referred to as ectosomes). Due to lack of specificity of EV markers and overlap in size between these categories, they are currently classified on

36 the basis of their physical characteristics into small EVs (sEVs; up to or around 100 nm,
37 encompassing both exosomes and small ectosomes) and large EVs (IEVs; above 100 nm) [2].
38 Both EV subtypes carry a variety of proteins, lipids and genetic material that can be transferred
39 to local and distant target cells and trigger a broad range of cellular activities and biological
40 responses to modulate their fate and behavior [3]. They are now increasingly viewed as
41 important mediators of intercellular communication in numerous physiological and
42 pathological processes, including development, immune responses, viral pathogenesis,
43 pregnancy, and cancer progression [1].

44 During pregnancy the placenta plays a pivotal role ensuring the transfer of nutrients
45 and oxygen between mother and fetus. This is achieved by an intimate communication
46 mediated by soluble factors but also by means of EVs [4–6]. The different placental cell types
47 (i.e., syncytiotrophoblasts, cytotrophoblasts, extravillous cytotrophoblasts) release a wide
48 range of EVs (small and large) into maternal circulation and fetal compartments (amniotic fluid
49 and fetal circulation), which gradually increase in concentration during pregnancy and stop
50 after delivery [5,7–9]. However, to avoid possible confusion and for simplification, we will use
51 the general term trophoblastic EVs in this review article. Placental-derived EVs play key roles
52 in regulating maternal-fetal communication in both normal and pathologic conditions
53 including gestational diabetes mellitus (GDM) [10], preeclampsia (PE) [11], and during viral
54 infections [12] (Figure 1).

55 The nature of this communication appears to be dependent on specific proteins and
56 microRNAs (miRNAs) enclosed and conveyed by placental EVs (Table 1). Among others,
57 placental EVs harbor the placental alkaline phosphatase (PLAP), an integral membrane protein
58 (enzyme) unique to the placenta [5]. They also contain proteins with immunological functions,
59 such as human leukocyte antigen G (HLA-G), MHC class I chain-related protein A and B (MIC-
60 A and -B), and syncytin-1, that respectively modulate maternal immune tolerance and reduce
61 the secretion of proinflammatory cytokines to protect fetal tissue from maternal immune cell
62 attack and prevent fetal rejection [13–15]. Placental EVs also contain functional placenta-
63 specific miRNAs, including the C19MC cluster of miRNAs [16,17] that confers viral resistance
64 to recipient cells [12]. However, in pathological pregnancies, the release of placental EVs was
65 shown to be affected by the microenvironment to which cells are exposed. Studies have also
66 demonstrated that changes in the microenvironment alter not only placental EV secretion,
67 but also their associated cargos and their bioactivity.

68 In this review, we describe current knowledge and gaps regarding placental EVs and
69 their roles in normal pregnancy and selected examples of gestational complications.

70

71 **Placental EVs in physiological pregnancy**

72 Placenta establishment

73 The success of human pregnancy highly depends on very early developmental processes, among which
74 the placenta establishment is crucial. After implantation of the blastocyst into the endometrium, the
75 outer layer of the embryo's trophoderm begins to invade maternal endometrium and differentiates

76 into syncytiotrophoblasts and cytotrophoblasts (villous and extravillous) [47]. These primitive
77 structures will develop into a mature placenta allowing exchanges between maternal blood and
78 embryo/fetal blood. The placenta itself evolves during pregnancy allowing an adaptive balance of the
79 utero-placental-fetal circulation according to the age of pregnancy.

80 Trophoblastic EVs have been shown to contribute locally to maternal-embryo communication.
81 Through their miRNA content, trophoblastic EVs modify the transcriptome of maternal endometrial
82 cells, including pathways essential for placental invasion (extracellular matrix remodeling), thereby
83 facilitating subsequent placental development [48,49]. Trophoblastic EVs also activate signaling
84 pathways necessary for successful invasion of the extravillous trophoblast (including matrix
85 metalloproteinases and mitogen-activated protein kinases) [50]. Control of oxygen (O₂) levels is also
86 crucial for the efficiency of the implantation/invasion process in humans. Under conditions of reduced
87 O₂ levels (e.g., hypoxia), the bioactivity of trophoblastic EVs is altered, promoting increased
88 proliferation and migration of extravillous cytotrophoblasts [51]. In addition to these local effects,
89 placental EVs in the maternal circulation modulate endothelial cell migration and may contribute to
90 the vascular adaptation of women to pregnancy state (including hypervolemia) [52]. Placental EVs may
91 also be involved in physiological pregnancies to limit adverse outcomes such as the pro-inflammatory
92 environment caused by air pollution, therefore playing a major potential role is maintaining healthy
93 fetal development [53].

94

95 Immune tolerance

96 Since the fetus and its placenta are considered as a semi-allogenic graft within the maternal host during
97 a natural conception, complex interactions operate at the maternal-fetal interface to allow adaptation
98 and tolerance of the maternal immune system [54–56]. Among the various mechanisms involved,
99 many roles of placental EVs have been documented *via* their transfer to maternal immune cells [8,57–
100 62], at a local level or by disseminating in the maternal circulation towards systemic lymphoid cells.
101 Placental EVs have been described to act mostly on Natural Killer (NK) cells [34,37,63], monocytes,
102 macrophages [21,22,25] and T cells [23,24,34,35,38], notably via their internalization and the delivery
103 of associated components.

104 They act mainly by promoting the suppression of maternal immune function at different levels.
105 Hence, several ligands of the NKG2D activating receptor of NK cells are released by trophoblastic sEVs,
106 including MIC-A and -B [64] and ULBP1-5 [34]. Interaction of NKG2D with sEVs harboring these ligands
107 leads to its down regulation at the surface of NK cells, CD8 and $\gamma\delta$ T cells, inhibiting their cytotoxic
108 activity *in vitro* [34]. Similarly, another NK activating receptor, NKp30, is suspected to be inhibited by
109 sEVs presenting B7H6, an endogenous ligand of NKp30, widely expressed by trophoblasts, which may
110 act as a decoy substrate [37]. Importantly, placental sEVs also carry the pro-apoptotic death
111 messengers FasL (Fas ligand) and TRAIL at their surface, inducing apoptosis of T cells and peripheral
112 blood mononuclear cells (PBMCs) *in vitro* [35], as well as the immunomodulatory molecule PD-L1,
113 responsible for down regulating the signaling CD3- ζ molecule at the cell surface, inhibiting T cell
114 activation [38]. Two other trophoblastic proteins carried by placental sEVs exert immunomodulatory
115 functions on T cells: Syncitin-2, by decreasing the secretion of Th1 cytokines, by activating PBMCs [24]
116 and HSPE1, and by promoting the expansion and differentiation of T reg *in vitro* [23]. Finally, circulating
117 monocytes are also down modulated by trophoblastic sEVs, notably via the action of miR-29a-3p,
118 promoting the expression of PD-L1 on their surface, which will further favor systemic immune
119 tolerance [25].

120 In addition to promoting maternal immune tolerance, EVs play an important role in providing
121 a mild inflammatory environment conducive to tissue remodeling and embryo implantation [65,58]. In
122 these pro-inflammatory events, placental EVs also act at different levels, as a chemoattractant for
123 monocytes *in vitro* [22], inducing their production of pro-inflammatory cytokines [22,21], and also
124 promoting the secretion of decidual cell of immune modulators via TNF α [26]. In addition, when
125 incubated with monocytes and B cells, placental IEVs induce TNF α , MIP-1 α , IL-1 α , IL-1 β , IL-6, IL-8
126 cytokines release [30]. Finally, placental IEVs have also been shown to activate neutrophils by
127 triggering the generation of extracellular traps in the intervillous space [31].
128

129 Antiviral role

130 The remodeling of the immune system at the maternal-fetal interface could favor the
131 development of viral and bacterial infections during this period of immunomodulation.
132 However, several mechanisms limit fetal rejection while simultaneously avoid pathogen
133 infection. These include the presence of immune cells from the maternal decidua, the
134 selective physical barrier represented by the placenta, but also the presence of a chemical
135 barrier that establishes a hostile environment for pathogens, notably provided by the
136 secretion of cytokines, interferons and placental EVs [66,67].

137 It has been widely proposed that placental EVs exhibit antiviral function [68,69].
138 Delorme-Axford *et al.* have proposed an EV-mediated transfer of antiviral resistance from
139 primary human trophoblasts to non-placental cells infected with a large variety of viruses from
140 different families [12]. This phenomenon was attributed to a family of 46 miRNAs, all deriving
141 from the largest miRNA cluster found on chromosome 19 (*i.e.* the C19MC cluster), that is
142 exclusively expressed in the placenta. This primate-specific miRNA cluster, highly enriched in
143 trophoblastic EVs, confers viral resistance by inducing autophagy once delivered to recipient
144 cells [70,17,71]. Although the miRNA profile was similar between the different EV populations,
145 the antiviral effect was specifically attributed to sEVs [28]. sEV from pregnant women's
146 plasma, abundantly found in maternal blood, and enriched in C19MC cluster also conferred
147 this antiviral resistance [28]. Some miRNAs in particular seem to be responsible for this
148 resistance (*i.e.* miR-517-3p), either because they are abundantly expressed in EVs, or because
149 they display an antiviral effect when expressed ectopically [72]. It should be noted, however,
150 that all these studies were carried out in models of term placenta. Whether the antiviral
151 resistance also occurs at the beginning of pregnancy remains to be determined.

152 Taken together, this evidence indicates that placental EVs display widespread effects on
153 normal pregnancy development. Placental EVs and associated content, once released into
154 target cells, regulate tissue remodeling for embryo implantation, the balance between
155 maternal tolerance towards the fetus and placenta, and pro-inflammatory events allowing
156 implantation and maintenance of the embryo. They also play a pivotal role in antiviral
157 protection, explained at least in part by their miRNA content, mainly from the C19MC cluster.

158

159 **Placental EVs in pathological pregnancy**

160 Gestational diabetes mellitus

161 Gestational diabetes mellitus (GDM) is a frequent metabolic disorder during pregnancy,
162 affecting around 15% of pregnant women [73]. It is defined as an abnormality of glucose
163 metabolism occurring or detected for the first-time during pregnancy. GDM is associated with
164 pronounced insulin resistance and hyperglycemia in mother and fetus, leading to risks of
165 gestational hypertension, premature delivery, fetal macrosomia amongst others, and a higher
166 risk of developing type 2 diabetes as well as cardiovascular diseases several years after
167 delivery for the mother and her offspring [74–76].

168 During GDM, placental EVs have been shown to be dysregulated, in term of
169 abundance, composition and function compared to normal pregnancies [8,59,77,78]. First, the
170 concentration of circulating total and placental sEVs is higher by nearly two-fold in maternal
171 circulation during GDM pregnancies compared to healthy pregnancies [39]. Second, miRNA
172 composition is also modified in circulating sEVs, notably miRNA involved in insulin regulation
173 and glucose pathways [40,43]. Placental EVs secreted from tissue explants also show a
174 dysregulation of their miRNA content, with gene ontology analysis indicating alterations in
175 pathways regulating cell migration and carbohydrate metabolism [36]. In term of protein
176 content, a proteomic study identified 78 differentially expressed proteins in GDM-derived
177 sEVs isolated from maternal circulation compared to healthy pregnant women [41]. These
178 differentially regulated proteins are primarily associated with energy production,
179 inflammation, and metabolism. It has also been shown that trophoblastic sEVs express 8-fold
180 more DiPeptidyl Peptidase IV (DPPIV) during GDM than in healthy pregnancy, an expression
181 that is retrieved in peripheral plasma [32].

182 These changes in miRNA and protein content in sEVs during GDM pregnancies impinge
183 on their biological functions. For example, placental sEVs from GDM women decrease insulin-
184 stimulated migration and glucose uptake in primary skeletal muscle cells from healthy patients
185 [36]. sEVs isolated from the plasma of GDM pregnant women induce insulin resistance and
186 glucose intolerance when injected in mice [42]. The increase of DPPIV expression observed on
187 GDM sEVs also favors insulin-resistance by decreasing circulating glucagon-like peptide-1 [32].
188 Finally, first-trimester trophoblastic sEVs have been shown to induce the release of cytokines
189 (IL-4, IL-6, IL-8, interferon- γ , and TNF- α) from endothelial cells upon glucose incubation [27].
190 Thus, placental EVs in the context of GDM may contribute to the pathophysiology of the
191 disease at multiple levels, targeting different cell types and functions.

192 Recently, studies performed on urinary sEVs report a specific increase in the damages
193 associated molecular pattern S100A9 protein at the 20th week of pregnancy [45] and a
194 decrease in the level of several C19MC miRNAs at the third trimester of gestation during GDM
195 compared to healthy pregnancy [46], indicating that urinary sEVs could be interesting sources
196 of biomarkers for GDM.

197 Pre-eclampsia

198 In early pregnancy, abnormalities during trophoblastic development and the implantation process can
199 lead to pre-eclampsia (PE). PE is characterized by systemic maternal symptoms (high blood pressure,
200 proteinuria) that can lead to eclampsia and maternal death if untreated. The lack of blood flow

201 between mother and fetus can also induce intra uterine growth restriction and other adverse effects
202 on the fetus [79]. As the exact etiology of PE remains unknown, treatments are mainly symptomatic
203 as they are not specific to this disease. In addition, there is a high risk of very rapid deterioration of the
204 maternal state and biomarkers to monitor the evolution of placental condition during PE are lacking.
205 In this context, placental EVs have been extensively studied in PE both in disease pathophysiology and
206 as potential biomarkers.

207 During PE, the total load of placental EVs increases and their composition is altered compared
208 with EVs from control pregnant women [80]. Trophoblastic EVs from patients with PE are enriched in
209 active neprilysin compared to control patients, which may contribute to the maternal hypertension
210 observed during PE [81]. The overall lipidomic profile of trophoblastic EVs is also altered during PE with
211 differences in certain lipid classes involved in oxidative stress, apoptosis, immune response, and
212 coagulation [82]. Placental EVs from pregnant women with PE appear to imbalance macrophage
213 polarization compared to healthy pregnancies and may therefore be an “self-promoter” of pre-
214 eclamptic state in patients with PE [83].

215 In an animal model, the administration of placental EVs purified from peripheral blood of
216 pregnant women with PE induced PE-like disease in pregnant mice with vascular defects including
217 disruption of the vascular endothelium and vasoconstriction [84]. Awoyemi *et al.* recently described
218 that trophoblastic EVs purified by lobal perfusion of late PE placenta limit the pro-inflammatory
219 immune response compared to healthy pregnancy and early PE trophoblastic EVs [85]. This suggests
220 that the disruption of the balance between pro/anti-inflammatory process evolves during pregnancy.
221 Placental EVs are also found in amniotic fluid where they participate in maternal-fetal communication
222 [86]. EVs from amniotic fluid during PE show an altered phenotype compared to healthy pregnancy
223 and display an antiangiogenic effect that may play a role in the development of PE [44].

224 Placental EVs are altered in amount and composition during PE [11,87]. The miRNA
225 composition of placental EVs found in the maternal blood of patients with pre-symptomatic PE who
226 subsequently developed PE is different from that of the control group of healthy pregnant women.
227 This prospective study identified miRNA that may be candidates for the development of biomarkers to
228 identify pregnant women at high risk for developing PE [88]. This active area of research is currently
229 identifying miRNA candidates and analyzing the expression profile of placental EVs in the maternal
230 circulation to develop non-invasive PE biomarker for diagnosis and monitoring [89,90].

231 232 Pro-microbial

233 Infections during pregnancy are common and some can lead to serious sequelae for both mother and
234 fetus [91–93]. In particular, the pathogens found under the acronym TORCH (*Toxoplasma gondii*, other
235 (Zika virus, *Listeria monocytogenes* (*Lm*) ...), Rubella virus, human Cytomegalovirus (hCMV), Herpes
236 Simplex virus) include viruses, bacteria and parasites involved in congenital infections. Transmission
237 occurs mainly vertically across the placenta, and placental EVs participate in the dissemination of the
238 pathogen in several infections and are altered in quantity, composition, and functions.

239 One example is congenital hCMV infection, which is the most common cause of infectious
240 deafness and brain malformations, affecting 1% of live births [94]. Our team characterized EVs from
241 first trimester placental tissue explants infected with hCMV and revealed some modifications of their
242 surface markers [33]. Moreover, protein content of EVs isolated from hCMV-infected cytotrophoblasts
243 was modified towards a proviral phenotype, which correlated with their ability to potentiate the
244 infection of naïve recipient fetal cells [18]. A similar phenomenon has been described in the context of
245 listeriosis infection in the work of Kaletka *et al* [19]. *Lm* is a Gram-positive food borne bacterium with

246 placental tropism, which can in the most serious cases lead to severe infections of the newborn,
247 premature deliveries or even fetal death [95,96]. The infection of trophoblast stem cells by this
248 pathogen lead to a modification of the protein and RNA content of the EVs derived from these cells
249 [19]. In addition, the functional evaluation of these EVs showed an increased activation of
250 macrophages, which were then in a pro-inflammatory state and more susceptible to infection by this
251 bacterium. These two different examples thus illustrate how pathogens can impact on placental EV
252 composition, which finally favor their dissemination, locally but also toward the fetal compartment.

253 Other examples are found in the literature, especially concerning Zika, a Flavivirus causing
254 congenital infections that can lead to serious neurosensory sequelae in the fetus [97]. Trophoblastic
255 EVs see both their size and their composition modified, particularly in terms of proteins where the
256 infection of the cells from which the EVs originate leads to an enrichment in mainly non-structural viral
257 proteins [20]. Another team demonstrated by histopathology, carried out on human placentas from
258 pregnant women naturally infected with Zika, an enlargement of the vesicles and apoptotic bodies of
259 the syncytiotrophoblasts, associated with an absence of normal secretion of IEVs [98].

260 Altogether, these different data demonstrate the importance of placental EVs during
261 pregnancy pathologies. Dysregulation of their secretion and/or content is observed in most, if
262 not all, gestational diseases. The question of whether this dysregulation is a consequence of
263 the pathology or if it contributes to the pathogenesis of the disease remains to be decipher.
264 Finally, the fact that these dysregulated EVs are found in abundance in maternal serum during
265 gestation make them interesting candidates as non-invasive biomarkers for pregnancy
266 pathologies.

267

268 **Perspectives**

269 The field of placental EVs is expanding, with increasing interest and a growing number
270 of studies highlighting their pivotal role across physiological and pathological gestations.
271 However, some confusions in the studies and the lack of precise terminology make
272 interpretation and comparison of different reports more difficult. Clarification of these issues
273 in the near future will help advance not only the field of placental EVs but also the entire EV
274 field.

- 275 • Significant work is underway to define a clear nomenclature for discriminate between
276 the different EV subpopulations. Most of the available data have been conducted on
277 sEVs, but the literature has to be read with caution because some studies have not
278 used the proper terms to identified the specific EV subpopulation considered in the
279 study.
- 280 • Although placental EVs are mainly secreted by syncytiotrophoblasts, most studies do
281 not specify the parental/donor cell type secreting EVs. To sort out placental EVs most
282 authors focus on PLAP, syncytin-1 or C19MC miRNAs, which are specifically expressed
283 in placental tissue. However, EVs secreted by embryonic appendages (including amnion
284 and umbilical cord) are sometimes mistaken for placental EVs.

- 285
- One question remains unanswered regarding the functional role of EVs in pathological pregnancies: is it a simple consequence of organ dysfunction or do placental EVs contribute to pathogenesis?
- 286
- 287
- The deregulation of placental EVs secretion and composition observed in pathological pregnancies makes them promising biomarkers for the diagnosis, prognosis and monitoring of gestational diseases.
- 288
- 289
- 290

291

292

293 Legend

294 **Figure 1. Effects of placental EVs in both physiological and pathological pregnancies.** Large and small EVs are symbolized by circles in different shades of blue for the physiological pregnancies, red for the pathological pregnancies. Trophoblastic EVs can act in an autocrine manner on the placenta and in a paracrine manner on the fetal or maternal compartments to regulate different physiological processes indicated next to each compartment on the left of the figure, in blue boxes. They are also implicated in some pathological pregnancies, indicated in red boxes on the right of the figure. Created with Biorender.com.

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581 **Competing Interests**

582 The authors declare that there are no competing interests associated with the manuscript.

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