

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Involvement of extracellular vesicle-encapsulated miRNAs in human reproductive disorders: a systematic review

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Barranco, I., Salas-Huetos, A., Berlanga, A., Spinaci, M., Yeste, M., Ribas-Maynou, J. (2022). Involvement of extracellular vesicle-encapsulated miRNAs in human reproductive disorders: a systematic review. *REPRODUCTION FERTILITY AND DEVELOPMENT*, 34(11), 751-775 [10.1071/RD21301].

Availability:

This version is available at: <https://hdl.handle.net/11585/899291> since: 2022-12-13

Published:

DOI: <http://doi.org/10.1071/RD21301>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Isabel Barranco, Albert Salas-Huetos, Angel Berlanga, Marcella Spinaci,
Marc Yeste, Jordi Ribas-Maynou

**Involvement of extracellular vesicle-encapsulated miRNAs in
human reproductive disorders: a systematic review**

Reproduction, Fertility and Development 2022;34:751 - 775

The final published version is available online at:

<https://doi.org/10.1071/RD21301>

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

1 **Title**

2 Involvement of extracellular vesicle-encapsulated miRNAs in human reproductive
3 disorders: a systematic review

4

5 **Running title**

6 Extracellular vesicles miRNA in human reproduction

7

8 **Authors**

9 Isabel Barranco^{1,*}, Albert Salas-Huetos^{2,3,4}, Angel Berlanga^{2,3}, Marcella Spinaci¹, Marc
10 Yeste^{2,3}, Jordi Ribas-Maynou^{2,3,*}

11

12 **Affiliations**

13 ¹Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy.

14 ²Biotechnology of Animal and Human Reproduction (TechnoSperm), Institute of Food
15 and Agricultural Technology, University of Girona, Girona, Spain.

16 ³Unit of Cell Biology, Department of Biology, Faculty of Sciences, University of Girona,
17 Girona, Spain.

18 ⁴ Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA,
19 USA.

20

21 ***Corresponding authors:**

22 Dr. Jordi Ribas-Maynou.

23 Address: Biotechnology of Animal and Human Reproduction (TechnoSperm), Institute
24 of Food and Agricultural Technology, University of Girona, ES-17003 Girona, Spain.

25 Contact: jordi.ribasmaynou@udg.edu. Tel.: +34 972 419514; Fax: +34 972 418150.

26 ORCID: 0000-0002-9101-2044

27

28 Dr. Isabel Barranco.

29 Address: Department of Veterinary Medical Sciences, University of Bologna, IT-40064

30 Ozzano dell'Emilia, Bologna, Italy. +39 051 2097904. isabel.barranco@unibo.it. ORCID:

31 0000-0001-9873-814X

32

33 **Data availability statement**

34 Data generated during the current study are available from the corresponding author on
35 reasonable request.

36

37 **Funding**

38 The authors acknowledge the support from the Ministry of Science and Innovation, Spain

39 (Grant: IJC2019-039615-I, A.S-H.); the Regional Government of Catalonia, Spain

40 (Grant: 2017-SGR-1229, M.Y.); European Union's Horizon 2020 research and

41 innovation programme (Grant: H2020-MSCA-IF-2019-891382, I.B.) and European

42 Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-

43 Curie agreement No. 801342 (Grant: TECSPR-19-1-0003, J.R.M.).

44

45 **Conflicts of interest**

46 Marc Yeste is an Editor of Reproduction, Fertility and Development, but was blinded

47 from the peer review process for this paper.

48 **Acknowledgements**

49 Not applicable

50

51 **Summary text**

52 In a wide variety of biological processes, extracellular vesicles are essential players in the
53 regulation of cell-to-cell communication. The present work consists of a systematic
54 review of studies analyzing the involvement of micro-RNAs contained in extracellular
55 vesicles in various reproductive-related disorders, such as including infertility, pregnancy
56 complications or embryo development.

57 Abstract

58 In the last years, EVs have emerged as essential players in cell-to-cell communication,
59 particularly having an active regulating role in biological systems. Because reproductive-
60 associated processes are not exempt of this communication, multiple studies have been
61 devoted to this realm, focusing on gamete maturation, embryo implantation or fetal
62 development. The aim of the present review was to collect comprehensively and
63 systematically the evidence about the function of the microRNA(miRNA) encapsulated
64 in EVs isolated from different reproductive tissues or fluids in reproductive-related
65 diseases. Following PRISMA guidelines, we conducted a systematic search of the
66 literature published in MEDLINE-PubMed until the end of February 2021. After
67 selection, 32 studies were included in the qualitative review comparing the miRNA
68 expression profile in EVs between different pathological conditions. Most reports showed
69 the potential of the miRNAs carried by EVs to be used as putative biomarkers of
70 reproductive conditions and disorders, including pregnancy affections, disease
71 progression and quality of preimplantation embryos. The most relevant miRNAs were
72 found to be highly heterogeneous among studies, with some conflicting results. Further
73 research is thus warranted to address whether confounding factors, such as the methods to
74 isolate EVs and miRNAs, the fraction of EVs, the criteria of patient selection, the timing
75 of sample retrieval, or any other factor, may explain these inconsistencies between
76 studies.

77

78 **Keywords:** Extracellular vesicles, exosomes, microvesicles, microRNAs, reproduction,
79 reproductive disorders,

80

81

82 **Introduction**

83 Extracellular vesicles (EVs) were first described in the '80s (Trams *et al.* 1981), when
84 they were suggested to remove harmful or useless molecules in order to protect the cell
85 from an accumulation of waste (Johnstone *et al.* 1991). Recently, EVs have gained much
86 relevance due to their intrinsic capacity of loading different types of bioactive molecules
87 (proteins, lipids, and nucleic acids) and safely transporting them from donor to recipient
88 cells, participating in a complex process of crosstalk between distant cells (Zomer *et al.*
89 2010). This strategy of exchange and cell-to-cell communication is being nowadays
90 highly studied, with research showing that specific nucleic acid cargo (mainly messenger
91 RNA (mRNAs) and microRNAs (miRNAs)) inside EVs can effectively affect the
92 biological behavior of recipient cells. Even under disease conditions, EVs can act as
93 promoting or restraining modulators leading to modifications in protein production and
94 gene expression of the recipient cell (Valadi *et al.* 2007). The EVs are a heterogeneous
95 population of round-shaped, lipid bi-layered membrane vesicles secreted by most cells
96 into the extracellular space. Extracellular vesicles have been isolated from many body
97 fluids, including urine (Zhang *et al.* 2016), saliva (Aqrawi *et al.* 2017), blood, breast milk
98 (Galley and Besner 2020), and reproductive fluids, such as follicular fluid, amniotic fluid
99 and semen among others (Colombo *et al.* 2014; Foster *et al.* 2016; Machtinger *et al.*
100 2016).

101 Human reproduction is a complex process involving a wide variety of cell types
102 that require crosstalk to achieve an adequate regulation at molecular level in order to
103 perform their function. The EVs are proven to be involved in reproductive processes at
104 many levels, from gamete generation and maturation to embryo implantation, both in men
105 and women (Sullivan 2016;; Simon *et al.* 2018; Vyas *et al.* 2019; Baskaran *et al.* 2020;
106 Foot and Kumar 2021). Each reproductive tissue is known to release specific EVs, which

107 have an unique cargo with a particular function in both the male and female genital tract
108 (Machtinger *et al.* 2016; Andronico *et al.* 2019). Specifically, it has been reported that
109 the miRNA cargo of EVs (EV miRNAs) is involved in key processes such as gamete
110 maturation, embryo development, immune modulation and cell invasion (Sullivan *et al.*
111 2005; Bechoua *et al.* 2011; Pons-Rejraji *et al.* 2011; Vojtech *et al.* 2014). The transfer of
112 miRNAs from donor to recipient cells through EVs has been previously demonstrated,
113 thus conferring the ability of modifying their functions (Valadi *et al.* 2007). Previous
114 studies also suggested that EV miRNAs can be used to determine the quality of oocytes
115 or to help verify the positive or negative outcome of an *in vitro* fertilization (IVF) process,
116 thus being a potential biomarker for the prediction of IVF outcomes in humans (Martinez
117 *et al.* 2018). Finally, the identification of miRNA cargo in EVs has also been shown to
118 anticipate the progression of some reproductive-related diseases, such as polycystic ovary
119 syndrome (PCOS), preeclampsia or pre-term birth (Simon *et al.* 2018). While it is still
120 unclear whether the dysregulation of this EV miRNA cargo could be the cause or the
121 consequence of these disorders, future studies could uncover the potential roles of these
122 EV miRNAs and help us to draw specific biomarkers or even treatments (Xu *et al.* 2019).
123 In this systematic review, therefore, we will focus on the miRNA cargo of EVs related to
124 human reproductive biology and the consequences/causes of their dysregulation. Thus,
125 the objective is to comprehensively and systematically collect the updated data about the
126 role of miRNA carried by EVs in reproductive physiology, identifying the miRNAs
127 encapsulated in EV in different fluids that are related to pathological reproductive
128 processes. **Materials and methods**

129 The present systematic review was conducted following the guidelines of the Preferred
130 Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Liberati *et al.*

131 2009). The protocol was registered in the PROSPERO registry
132 (<http://www.crd.york.ac.uk/PROSPERO>; PROSPERO 2021 ID: CRD42021275747).

133

134 *Data sources and search strategy*

135 A systematic analysis of the available literature was conducted using the MEDLINE-
136 PubMed database (<http://www.ncbi.nlm.nih.gov/pubmed>), including published studies
137 until 28th February 2021, and a manual search of the reference list of retrieved articles.

138 In order to define inclusion and exclusion criteria, a PICOS (Population,
139 Intervention, Comparator, Outcome, Study) Table was designed prior to any search
140 (Table 1). Keywords were selected based on the PICOS table and were aligned with the
141 main objective of this work. The search strategy resulted from the combination of the
142 selected terms and was conducted in PubMed as follows: (miRNA OR miRNA profile
143 OR miRNA expression OR small RNA profile OR small RNA expression) AND
144 (reproduction OR reproductive OR fertility OR fertilization OR reproductive tissue OR
145 assisted reproductive technology) AND (extracellular vesicle OR exosome OR
146 microvesicle OR vesicle) AND (human or homo sapiens). We also applied a filter to meet
147 with inclusion criteria: Humans, English.

148 *Study selection and eligibility procedure*

149 Results obtained from PubMed were downloaded in *.txt* format using a standardized
150 extraction form that collected the following information: reference, digital object
151 identifier (DOI), publication year, title, abstract, authors and article type. An *Excel* file
152 was generated with all this information. All information was screened in parallel by two
153 authors (I.B. and A.B.) for eligibility and any discrepancies were re-evaluated together
154 with a third author (J.R-M.).

155 Selection of studies started once all records were annotated in the database; article

156 types declared as non-eligible were directly excluded. The second stage in study selection
157 was based on title and abstract screening, excluding those articles that did not meet the
158 eligibility criteria. Thereafter, the full text of all selected articles was downloaded and
159 screened for a third step of exclusion, that was conducted to obtain the final list of selected
160 articles.

161 For a study to be eligible, it had to have been performed in humans (males and/or
162 females), so animal studies were ineligible. The outcome was also an eligibility criterion,
163 each study being necessarily aimed at characterizing miRNA in EVs and/or including
164 data about miRNAs dysregulation (up/down) in human reproductive disorders, thus
165 comparing pathological *vs.* non-pathological conditions. Hence, reports analyzing
166 miRNAs not contained within EVs, or descriptive studies were excluded. Regarding the
167 type of articles, research articles, meta-analyses, observational studies, cross-sectional,
168 comparative and longitudinal studies were included, whereas letters, commentary
169 articles, review articles and systematic reviews were excluded.

170

171 *Data extraction for systematic review*

172 After selecting the articles on the basis of their title/abstract, the full text of each selected
173 study was analyzed and the following information was extracted: author/s, year of
174 publication, journal, title of the article, participant conditions, outcomes related to the
175 miRNA encapsulated within EVs, and major findings about up/down regulations of these
176 miRNAs related to reproductive processes, in both men and women.

177

178 **Results**

179 *Identification and selection of the studies*

180 After the initial search carried out using the PubMed database, 302 articles were recorded
181 (Figure 1). Among these 302 records, 87 were immediately excluded, as they were
182 narrative or systematic reviews. A further title and abstract screening was performed,
183 excluding 162 records that did not meet the inclusion criteria. The remaining 53 articles
184 were downloaded for full text eligibility assessment; 21 were excluded due to the
185 following reasons: descriptive studies without comparison between pathological and non-
186 pathological conditions (n = 9); not associated to EVs (n = 6); not related to reproductive
187 biology (n = 2); not performed in humans (n = 2) or not written in English (n=2). We,
188 therefore, obtained a final list of 32 studies that were declared eligible as per the inclusion
189 and exclusion criteria defined in the PICOS Table for this systematic review (Table 1).

190

191 *Selected studies overview*

192 Studies selected for analysis, which are summarized in Table 2, were organized on the
193 basis of their specific aims and following the previously defined criteria.

194 Studies included had a comparative objective, i.e., subjects displaying
195 abnormal/pathological reproductive condition vs. normal/health (Table 2). Out of the 32
196 studies included, two were focused in men and the other 30 investigated female-related
197 reproductive disorders. The male-factor studies examined the expression profile of EV
198 miRNA in seminal plasma, assessing the potential relationship of miRNAs encapsulated
199 within EVs with oligoasthenozoospermia/azoospermia. Among the studies focused in
200 female factors, one examined the differential miRNA expression profile between EVs
201 released from endometriotic and normal endometrial tissue; 15 examined the differential
202 EV miRNA expression profile in blood plasma between healthy and pregnancy-related
203 complications such as preterm birth (n = 3), gestational diabetes (n = 1), preeclampsia (n
204 = 10) and fetal growth (n = 1); three examined the differential expression profile in

205 placenta-derived EV miRNA between healthy and pregnancy-related complications, such
206 as gestational diabetes (n =1), and preeclampsia (n = 2); nine examined the differential
207 miRNA expression profile in follicular fluid derived EVs, three in normal and PCOS-
208 pregnancies, two in patients with different age, one in patients with different body mass
209 index and three in oocytes or pre-implantation embryos of different quality; one examined
210 miRNAs in EVs isolated from uterine fluid in order to find receptivity associated
211 biomarkers; and one article examined the differential miRNA expression profile of EV
212 isolated from peritoneal fluid between endometriosis and healthy women.

213

214 **Discussion**

215 The present study systematically reviewed the available literature about the miRNAs
216 transported by EVs and their role under pathological conditions, providing
217 comprehensive and useful information that not only could be essential to understand the
218 crosstalk between separate cell types in reproductive biology, but could also point out to
219 the upregulation or downregulation of EVmiRNAs caused by different reproductive
220 disorders. As a wide range of affectations was identified, the miRNAs carried by the EVs
221 involved in different reproductive processes will be discussed separately in this section.

222

223 *Role of miRNAs carried by EVs in male reproductive physiology*

224 Because infertility due to the male factor affects half of infertile couples (Leaver 2016),
225 new, non-invasive biomarkers are needed to predict the chances of having a successful
226 pregnancy in these couples. Growing evidence points to seminal EVs as key modulators
227 of sperm physiological processes, including sperm maturation, motility, capacitation, and
228 acrosome reaction, influencing the fertilization process (Ronquist 2012; Sullivan and
229 Saez 2013; Baskaran *et al.* 2020; Wu *et al.* 2020). Two studies included in this systematic

230 review (Abu-Halima *et al.* 2016; Barceló *et al.* 2018) were focused on the analysis of the
231 miRNAs contained in seminal plasma EVs and aimed at uncovering the causes and
232 biomarkers of oligo/azoospermia. The assessment of more than 600 mature miRNAs in
233 these two studies showed that several miRNAs were dysregulated in azoospermic men;
234 specifically, 36 in Abu-Halima *et al.* (2016) and 60 in Barceló *et al.* (2018). Surprisingly,
235 while four of these dysregulated miRNAs (miR-23b, miR-21, miR-363 and miR-96) were
236 identified in both studies, they exhibited an opposite pattern. Differences in the RNA
237 isolation method, miRNA analysis or patient selection between these two studies could
238 contribute to explain these inconsistent results.

239 Among the dysregulated miRNAs encapsulated within seminal plasma EVs, Abu-
240 Halima *et al.* (2016) found a higher expression of miR-765 and miR-1275 and lower
241 expression of miR-15a in oligoasthenozoospermic men. Interestingly, bioinformatics
242 analysis predicted that the genes targeted by these miRNAs were involved in Ras, ErbB,
243 MAPK, cAMP, PI3k-Akt, Hedgehog and Wnt signaling pathways. As all these biological
244 pathways have been described to be involved in spermatogenesis (Vojtech *et al.* 2014),
245 one could suggest that the oligozoospermia observed in these patients would result from
246 an impaired spermatogenesis. In addition, Barceló *et al.* (2018) suggested that some
247 miRNAs (miR-31-5p, miR-539-5p and miR-941) encapsulated within seminal plasma
248 EVs could establish the origin of azoospermia. Moreover, these miRNAs were found to
249 be expressed in testis, epididymis and prostate, suggesting their involvement in cell-to-
250 cell communication occurring alongside the male genital tract.

251

252 *Role of miRNAs carried by EVs in female reproductive processes*

253 Endometriosis

254 It is thought that women suffering from endometriosis may have immune dysfunctions
255 that can interfere with a correct clearing of the lesions caused by abnormal tissue growth
256 (Giudice 2010). Two studies assessing this dysfunction were included in the present
257 review (Chen *et al.* 2019; Khalaj *et al.* 2019), showing that women suffering from
258 endometriosis carry a unique miRNA profile within EVs in endometriotic tissues,
259 peritoneal fluid and blood plasma. Bioinformatics analysis showed that some
260 downregulated miRNAs, such as miR-27a and miR-375, had binding sites for *SERPINA1*,
261 *PDGFA* and *THBS1*, which are essential genes involved in embryonic development,
262 angiogenesis, cell proliferation and differentiation (Khalaj *et al.* 2019). Also, other
263 upregulated miRNAs, such as miRNA-451a, miRNA-1908 and miRNA-130b, were
264 found to alter immune cells, such as macrophages and Treg, contributing to an abnormal
265 immunological microenvironment promoting endometriosis (Chen *et al.* 2019). Related
266 to miRNA-451a, it was upregulated in both studies (Khalaj *et al.* 2019; Chen *et al.* 2019)
267 and was downregulated in EV isolated from blood plasma of women with preeclampsia
268 (Truong *et al.* 2017) and from chorionic villous explants of women with gestational
269 diabetes compared to women with normal pregnancy (Nair *et al.* 2018).. Similarly, in EV
270 isolated from peritoneal fluid from women with pregnancies complicated by
271 endometriosis, miRNA-505-5p was upregulated (Chen *et al.* 2019), which was also
272 upregulated in EV isolated from blood plasma from women with preterm birth delivered
273 (Fallen *et al.*, 2018). These findings suggest the putative key role of miRNA-451a and
274 miRNA-505-5p encapsulated in EVs in female reproductive disorders.

275

276 Reproductive aging

277 Infertility is constantly raising in the last years, and the advancement of maternal age is
278 known to be one of the main factors leading to that increase (Carson and Kallen 2021).

279 Regarding the ageing processes taking place in women, two studies were focused on
280 comparing the miRNA expression profile of EVs isolated from the follicular fluid
281 between two age groups of women (older and young) (Diez-Fraile *et al.* 2014; Battaglia
282 *et al.* 2020). Results of these two studies showed that several miRNAs transported by the
283 EVs present in the follicular fluid were differentially upregulated and downregulated in
284 both groups, but none of them was common between both studies. Diez-Fraile *et al.*
285 (2014) found three EV miRNAs that were solely expressed in one of the groups: one in
286 younger women (miR-21-5p) and two in older women (miR-190b and miR-99b-3p).
287 These identified miRNAs were found to be involved in TP53 signaling pathways, heparan
288 sulfate biosynthesis, and extracellular matrix-receptor interaction, influencing oocyte
289 maturation, stress response and vesicle release. These pathways are also known to be
290 related to fertility (Diez-Fraile *et al.* 2014). Additionally, the increased level of apoptosis
291 in granulosa cells that was seen in older women was also found to be related to the
292 downregulation of miR-21-5p and to the upregulation of miR-134 (Krysko *et al.* 2008),
293 thus indicating that apoptotic processes could also be predicted through these miRNA.
294 Finally, miR-16-5p, which is downregulated in old women (Battaglia *et al.* 2020), was
295 reported to be downregulated in women with poor embryo quality (Machtinger *et al.*
296 2017), showing a relationship between these two conditions.

297

298 Polycystic ovarian syndrome (PCOS)

299 Polycystic ovarian syndrome usually courses with hyperandrogenism, obesity, polycystic
300 ovarian morphology, insulin resistance and/or anovulation, thus affecting oocyte quality.
301 Three studies included in this review (Sang *et al.* 2013; Hu *et al.* 2020; Rooda *et al.* 2020)
302 compared the expression profile of the EV-miRNAs present in the follicular fluid between
303 women suffering from PCOS and those not suffering from that disease. The three studies

304 demonstrated that several miRNAs transported by EVs were involved in amino acid and
305 glycosaminoglycan biosynthesis, and that carbon and monocarboxylic metabolism was
306 dysregulated in PCOS patients (Sang *et al.* 2013; Hu *et al.* 2020; Rooda *et al.* 2020). In
307 these three studies, the main over- and under-expressed miRNAs (Table 2) were proposed
308 to be potential early biomarkers of this disorder; however, their utility remains
309 controversial, as opposite results were found for two miRNA (miR-10a-5p and miR-
310 200c-3p), which were down- (Hu *et al.*, 2020) and upregulated (Rooda *et al.*, 2020),
311 respectively. In this regard, it can be hypothesized that differences could be due to the
312 method used to isolate EVs (ultracentrifugation for Hu *et al.*, 2020 *vs* chromatography for
313 Rooda *et al.*, 2020), but one has to take into account that other factors, such as the RNA
314 isolation method, differed between these studies. Moreover, variables such as the use of
315 different patient/donor cohorts may also explain such differences. For all these reasons,
316 more research needs to be conducted to reduce these uncertainties, before accepting the
317 clinical utility of these miRNAs.

318

319 *Role of miRNAs carried by EVs in pregnancy-related processes*

320 Embryo/Oocyte quality

321 While the success rates of single embryo transfer following ICSI in humans have been
322 improved in the last decades, mounting evidence supports that they have reached a plateau
323 (European IVF-monitoring Consortium (EIM) for the European Society of Human
324 Reproduction and Embryology (ESHRE) *et al.*, 2020). Despite the usefulness of classical
325 embryo parameters, many efforts are focused on uncovering potential biomarkers that
326 could have better predictive ability upon embryo implantation and the achievement of life
327 birth (Gardner and Balaban 2016). In this regard, three studies included in our review
328 aimed at comparing the follicular fluid-derived EV miRNA cargoes between top- and

329 poor-quality oocytes/preimplantation embryos (Machtinger *et al.* 2017; Martinez *et al.*
330 2018; Zhang *et al.* 2021). The identification of miRNAs encapsulated in EV led to the
331 finding of several dysregulated miRNAs in the follicular fluid of oocytes that failed to be
332 fertilized. The dysregulated miRNAs from embryos with fertilization failure reported in
333 the studies (Table 2) were predicted to target genes involved in organ development,
334 reproductive system diseases and systemic abnormalities. In the same way, miRNA
335 dysregulation was identified in follicular fluid EVs isolated from follicles that led to poor-
336 quality embryos. These miRNAs were found to be involved in follicular growth,
337 regulation of oocyte meiosis, cellular signaling and ovarian function pathways (Martinez
338 *et al.* 2018). All these findings suggest that follicular fluid EV-borne miRNAs could be
339 crucial for proper embryo development and fertilization, and could be used as potential
340 biomarkers to predict embryo quality and pregnancy success.

341

342 Preeclampsia

343 Preeclampsia is one of the most prevalent pregnancy-related diseases affecting women
344 worldwide, and is defined as an onset of hypertension during the second half of pregnancy
345 (Kuklina *et al.* 2009). This disease leads to an increase in oxidative stress and underlies
346 the development of systemic endothelial dysfunction, which results in the characteristic
347 clinical symptoms in later stages of the disease. Twelve studies included in this review
348 were focused on investigating the EV-borne miRNAs, most of them isolated from blood
349 plasma, in order to find putative early biomarkers aimed to reduce the prevalence and
350 severity of this disease and to better understand its progression and pathophysiology
351 (Ospina-Prieto *et al.* 2016; Sandrim *et al.* 2016; Biró *et al.* 2017, 2019; Cronqvist *et al.*
352 2017; Salomon *et al.* 2017; Truong *et al.* 2017; Motawi *et al.* 2018; Hromadnikova *et al.*
353 2019; Pillay *et al.* 2019; Wang *et al.* 2020; Xueya *et al.* 2020).

354 In two studies from the same research group Biró *et al.* 2017, 2019, authors
355 purported that an upregulation of the miR-210-3p carried in EVs could be a preeclampsia
356 indicator in blood. This finding could not be confirmed in the study of Cronqvist, who
357 found similar levels among the studied groups. The predicted target genes related to miR-
358 210 are involved in cell proliferation and differentiation, apoptosis, angiogenesis and
359 metabolism. Based on these data, Lee *et al.* (2011) hypothesized that high levels of miR-
360 210 could lead to oxidative stress and placental mitochondria dysfunction through the
361 repression of Iron-Sulfur Cluster assembly enzyme (ISCU) protein, which leads to iron
362 accumulation in the mitochondria of trophoblast cells. The study by Wang *et al.* (2020)
363 investigated the miR-15a-5p carried by EVs and found that an elevated expression of this
364 miRNA could inhibit the proliferation of granulosa cells through downregulation of its
365 targeted gene, *CDK1*, which is involved in the PI3k-AKT-mTOR pathway (Borges *et al.*
366 2020). Related with this, it is worth mentioning that this pathway has been associated to
367 preeclampsia in rodents (Huang *et al.* 2020), which adds value to this potential biomarker.

368 Another study carried out by Sandrim *et al.* (2016) found that miR-376c-3p, miR-
369 19a-3p and miR-19b-3p were downregulated and miR-885-5p was upregulated in EVs
370 when preeclampsia patients and controls were compared. While the relationship between
371 miR-885-5p and this disorder remains unclear, the high prevalence of this miRNA in
372 preeclampsia patients suggests an intercellular communication role *via* targeting its
373 predicted gene targets, *CDK2* and *MCM5*, both involved in cell proliferation and survival
374 (Afanasyeva *et al.* 2011). Thus, the upregulation of this miRNA could lead to cellular
375 senescence and apoptosis (Huppertz *et al.* 2006), which are common features in
376 preeclampsia.

377 Finally, miR-141-3p, miR-525-5p, miR-376c-3p, miR-517c and miR-517a-3p
378 were found to be dysregulated in preeclampsia patients, and also in women with preterm
379 birth (Fallen *et al.* 2018), which would suggest that these disorders are related.

380

381 Preterm birth

382 While the initiation of parturition occurs when fetal development is completed and is
383 related to immune and feto-maternal endocrine changes in the uterine cavity (Mendelson
384 2009), labor timing is also surmised to be regulated by the miRNAs present in EVs
385 derived from placenta and umbilical artery. Related to this hypothesis, three studies
386 included in this review compared the miRNA expression profile in EVs isolated from
387 blood plasma and Primary Human Trophoblast (PHT) cells between women with preterm
388 and with full-term labors (Fallen *et al.* 2018; Menon *et al.* 2019; Yadava *et al.* 2021). A
389 dysregulation in the miRNA expression profile of EVs was found in preterm birth patients
390 compared to full-term pregnancies. Fallen *et al.* (2018) analyzed more than 500 miRNA
391 and indicated that nearly 50% belonged to the placental expression of *CI9MC*, which
392 reflects the overall health status in the placenta. The genes targeted by most of the
393 dysregulated miRNAs found in blood plasma of women who had preterm birth were
394 described to be related to cell proliferation and focal adhesion molecules, affecting PI3K,
395 AKT and VEGF signaling pathways (Fallen *et al.* 2018). Another study suggested that
396 the upregulation of miR-15b-5p in EVs released from PHT cells could be an interesting
397 biomarker for preterm birth (Yadava *et al.* 2021). Since the predicted target gene of miR-
398 15b-5p was *APLN*, its repression is known to upregulate proinflammatory cytokines in
399 the placenta, resulting in several processes regarding homeostasis, cardiovascular
400 function and regulating cell apoptosis and oxidative stress regulation (Briana and
401 Malamitsi-Puchner 2009). As previously stated, five miRNAs were commonly

402 dysregulated both in preterm birth and in preeclampsia, thus suggesting that both
403 affectations can be somehow related to them. These miRNAs encapsulated within EVs,
404 therefore, could be considered as putative biomarkers of these pathologies.

405

406 Gestational diabetes mellitus

407 Gestational diabetes mellitus is defined as glucose intolerance leading to maternal
408 hyperglycemia and hyper-insulinemia, and is diagnosed during pregnancy with absence
409 of previous type I or II diabetes mellitus (Feig *et al.* 2018). Two studies included in this
410 review investigated the differential miRNA expression profile of EVs isolated from blood
411 plasma and placental tissue between women with pregnancy complicated by gestational
412 diabetes and women with normal pregnancies (Nair *et al.* 2018; Gillet *et al.* 2019). Gillet
413 *et al.* (2019) identified 10 miRNAs upregulated in EVs isolated from blood plasma of
414 gestational diabetes patients; the bioinformatics analysis showed these miRNAs were
415 involved in glucose transport and insulin secretion and regulation in pregnant women,
416 affecting relevant pathways for gestational diabetes such as AMPK (insulin receptor
417 signaling pathway). Nair *et al.* (2018) identified 456 miRNAs in placental derived-EVs
418 and found 23 of them dysregulated between GDM patients and healthy women (nine
419 upregulated and 14 downregulated). The genes predicted to be targeted by miRNAs were
420 related to PI3/AKT signaling and glucose metabolism/insulin resistance pathways, which
421 regulated cell migration and carbohydrate metabolism. Finally, miR-197-3p was found to
422 be dysregulated in gestational diabetes, low fetal growth and women with preterm birth
423 (Rodosthenous *et al.* 2017; Nair *et al.* 2018; Menon *et al.* 2019), evidencing a possible
424 common physiopathology.

425

426 **Strengths and limitations**

427 It is a strength of our review the comprehensive collection of studies relating the miRNAs
428 transported by EVs to the different disorders affecting human reproduction. The
429 systematic approach contributes to this strength, as it was conducted following inclusion
430 and exclusion criteria that were defined prior to the literature search. Even though most
431 of the studies analyzed miRNAs through an *-omics* approach, thus obtaining up- and
432 downregulation for hundreds to thousands of genes, the present work may show a
433 limitation regarding the publication bias, as non-conclusive results could prevent
434 publication, either by the authors or by the journal Editors. Another limitation would be
435 that the search was conducted in a single database (MEDLINE-PubMed). While it is well
436 known that this database covers most of the published works in medical topics, the
437 inclusion of other search databases could have strengthened the retrieval of scientific
438 articles. Finally and importantly, the lack of consensus on EVs isolation method
439 undermines our ability to compare and integrate results from different studies focused on
440 the same reproductive disorder and to establish miRNAs encapsulated in EVs as specific
441 reproductive pathology-biomarker. In this sense, methodological-related differences in
442 the size, quantity, yield and composition of isolated EVs, and even in the miRNAs
443 encapsulated in EVs have been reported (Buschmann *et al.* 2018; Brennan *et al.* 2020).
444 For this reason, further studies are required to establish an accurate protocol for the
445 analysis of EV-borne miRNAs, particularly in reproductive fluids and tissues.

446

447 **Conclusions**

448 The amount of miRNAs found to be upregulated or downregulated in pathological
449 reproductive diseases compared to healthy individuals show the importance of EVs in
450 cell regulation, proving that they are involved in cell-to-cell communication and that play
451 key roles in the regulation of all reproductive processes, from gametogenesis (Ji *et al.*

452 2013; Barceló *et al.* 2018), to fertilization (Machtinger *et al.* 2017; Rooda *et al.* 2020), or
453 even during pregnancy (Salomon *et al.* 2017; Xueya *et al.* 2020). This regulating ability
454 of miRNAs could be due to the protective effect of EVs that prevent miRNAs from
455 degradation, allowing them to safely travel from donor to recipient cells. A highly
456 heterogeneous set of miRNAs, however, is usually observed in studies assessing similar
457 disorders, thus evidencing a lack of consensus in the method or kit used to isolate EVs,
458 the EV fraction studied, the RNA isolation method, the miRNA analysis method, criteria
459 of patient selection, and the biological fluid used or the sample timing. For this reason,
460 further studies are required to elucidate the differences between these factors. Finally,
461 further comprehensive understanding of the molecular mechanisms behind EVs
462 modulation is important, as biosynthesis of EVs to encapsulate therapeutic drugs can
463 allow generating novel therapeutic strategies for a high variety of affectations.

464

465 **Author contributions**

466 I.B. and JR-M conceived the study and performed the study design. A.B. and I.B.
467 performed the search and eligibility selection, systematic review analysis, interpreted
468 results and discussed results. A.B., I.B. and J.R-M. wrote the manuscript and revised the
469 manuscript. AS-H. and M.S. critically revised the manuscript. M.Y., I.B. and J.R-M.
470 conceived the study, interpreted and discussed the results, critically revised the
471 manuscript and approved the final version. All authors approved the final version and
472 provided substantial intellectual contributions.

473

474

475

476 **REFERENCES**

- 477 Abu-Halima M., Ludwig N., Hart M., Leidinger P., Backes C., Keller A., Hammadeh M.,
478 and Meese E. (2016). Altered micro-ribonucleic acid expression profiles of
479 extracellular microvesicles in the seminal plasma of patients with
480 oligoasthenozoospermia. *Fertil. Steril.* **106**, 1061-1069.e3.
481 doi:10.1016/j.fertnstert.2016.06.030
- 482 Afanasyeva E. A., Mestdagh P., Kumps C., Vandesompele J., Ehemann V., Theissen J.,
483 Fischer M., Zapatka M., Brors B., Savelyeva L., Sagulenko V., Speleman F.,
484 Schwab M., and Westermann F. (2011). MicroRNA miR-885-5p targets CDK2 and
485 MCM5, activates p53 and inhibits proliferation and survival. *Cell Death Differ.* **18**,
486 974–984. doi:10.1038/cdd.2010.164
- 487 Andronico F., Battaglia R., Ragusa M., Barbagallo D., Purrello M., and Di Pietro C.
488 (2019). Extracellular vesicles in human oogenesis and implantation. *Int. J. Mol. Sci.*
489 **20**,. doi:10.3390/ijms20092162
- 490 Aqrabi L. A., Galtung H. K., Vestad B., Øvstebø R., Thiede B., Rusthen S., Young A.,
491 Guerreiro E. M., Utheim T. P., Chen X., Utheim Ø. A., Palm Ø., and Jensen J. L.
492 (2017). Identification of potential saliva and tear biomarkers in primary Sjögren’s
493 syndrome, utilising the extraction of extracellular vesicles and proteomics analysis.
494 *Arthritis Res. Ther.* **19**, 1–15. doi:10.1186/s13075-017-1228-x
- 495 Barceló M., Mata A., Bassas L., and Larriba S. (2018). Exosomal microRNAs in seminal
496 plasma are markers of the origin of azoospermia and can predict the presence of
497 sperm in testicular tissue. *Hum. Reprod.* **33**, 1087–1098.
498 doi:10.1093/humrep/dey072
- 499 Baskaran S., Panner Selvam M. K., and Agarwal A. (2020). ‘Exosomes of male
500 reproduction.’ (Elsevier Inc.) doi:10.1016/bs.acc.2019.08.004

- 501 Battaglia R., Musumeci P., Ragusa M., Barbagallo D., Scalia M., Zimbone M., Lo Faro
502 M. J., Borzì P., Scollo P., Purrello M., Vento M. E., and Di Pietro C. (2020). Ovarian
503 aging increases small extracellular vesicle CD81+ release in human follicular fluid
504 and influences miRNA profiles. *Aging (Albany, NY)*. **12**, 12324–12341.
505 doi:10.18632/aging.103441
- 506 Bechoua S., Rieu I., Sion B., and Grizard G. (2011). Prostrasomes as potential modulators
507 of tyrosine phosphorylation in human spermatozoa. *Syst. Biol. Reprod. Med.* **57**,
508 139–148. doi:10.3109/19396368.2010.549538
- 509 Biró O., Alasztics B., Molvarec A., Joó J., Nagy B., and Rigó J. (2017). Various levels of
510 circulating exosomal total-miRNA and miR-210 hypoxamiR in different forms of
511 pregnancy hypertension. *Pregnancy Hypertens.* **10**, 207–212.
512 doi:10.1016/j.preghy.2017.09.002
- 513 Biró O., Fóthi Á., Alasztics B., Nagy B., Orbán T. I., and Rigó J. (2019). Circulating
514 exosomal and Argonaute-bound microRNAs in preeclampsia. *Gene* **692**, 138–144.
515 doi:10.1016/j.gene.2019.01.012
- 516 Borges E., Mulato M. G. F., Setti A. S., Iaconelli A., Geraldo M. V., and Braga D. P. de
517 A. F. (2020). Serum microRNA profiling for the identification of predictive
518 molecular markers of the response to controlled ovarian stimulation. *J. Bras.*
519 *Reprod. Assist.* **24**, 97–103. doi:10.5935/1518-0557.20190070
- 520 Brennan K., Martin K., FitzGerald S. P., O’Sullivan J., Wu Y., Blanco A., Richardson
521 C., and Mc Gee M. M. (2020). A comparison of methods for the isolation and
522 separation of extracellular vesicles from protein and lipid particles in human serum.
523 *Sci. Rep.* **10**, 1039. doi:10.1038/s41598-020-57497-7
- 524 Briana D. D., and Malamitsi-Puchner A. (2009). Reviews: Adipocytokines in normal and
525 complicated pregnancies. *Reprod. Sci.* **16**, 921–937.

- 526 doi:10.1177/1933719109336614
- 527 Buschmann D., Kirchner B., Hermann S., Märte M., Wurmser C., Brandes F., Kotschote
528 S., Bonin M., Steinlein O. K., Pfaffl M. W., Schelling G., and Reithmair M. (2018).
529 Evaluation of serum extracellular vesicle isolation methods for profiling miRNAs
530 by next-generation sequencing. *J. Extracell. Vesicles* **7**, 1481321.
531 doi:10.1080/20013078.2018.1481321
- 532 Carson S. A., and Kallen A. N. (2021). Diagnosis and Management of Infertility: A
533 Review. *JAMA* **326**, 65–76. doi:10.1001/jama.2021.4788
- 534 Chen Y., Wang K., Xu Y., Guo P., Hong B., Cao Y., Wei Z., Xue R., Wang C., and Jiang
535 H. (2019). Alteration of Myeloid-Derived Suppressor Cells, Chronic Inflammatory
536 Cytokines, and Exosomal miRNA Contribute to the Peritoneal Immune Disorder of
537 Patients With Endometriosis. *Reprod. Sci.* **26**, 1130–1138.
538 doi:10.1177/1933719118808923
- 539 Colombo M., Raposo G., and Théry C. (2014). Biogenesis, secretion, and intercellular
540 interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell Dev. Biol.*
541 **30**, 255–289. doi:10.1146/annurev-cellbio-101512-122326
- 542 Cronqvist T., Tannetta D., Mörgelin M., Belting M., Sargent I., Familiari M., and Hansson
543 S. R. (2017). Syncytiotrophoblast derived extracellular vesicles transfer functional
544 placental miRNAs to primary human endothelial cells. *Sci. Rep.* **7**, 1–14.
545 doi:10.1038/s41598-017-04468-0
- 546 Diez-Fraile A., Lammens T., Tilleman K., Witkowski W., Verhasselt B., De Sutter P.,
547 Benoit Y., Espeel M., and D’Herde K. (2014). Age-associated differential
548 microRNA levels in human follicular fluid reveal pathways potentially determining
549 fertility and success of in vitro fertilization. *Hum. Fertil.* **17**, 90–98.
550 doi:10.3109/14647273.2014.897006

- 551 European IVF-monitoring Consortium (EIM)‡ for the European Society of Human
552 Reproduction and Embryology (ESHRE) W., Wyns C., Bergh C., Calhaz-Jorge C.,
553 De Geyter C., Kupka M. S., Motrenko T., Rugescu I., Smeenk J., Tandler-Schneider
554 A., Vidakovic S., and Goossens V. (2020). ART in Europe, 2016: results generated
555 from European registries by ESHRE. *Hum. Reprod. open* **2020**, hoaa032.
556 doi:10.1093/hropen/hoaa032
- 557 Fallen S., Baxter D., Wu X., Kim T. K., Shynlova O., Lee M. Y., Scherler K., Lye S.,
558 Hood L., and Wang K. (2018). Extracellular vesicle RNAs reflect placenta
559 dysfunction and are a biomarker source for preterm labour. *J. Cell. Mol. Med.* **22**,
560 2760–2773. doi:10.1111/jcmm.13570
- 561 Feig D. S., Berger H., Donovan L., Godbout A., Kader T., Keely E., and Ma R. S. (2018).
562 2018 Clinical Practice Guidelines Diabetes and Pregnancy Diabetes Canada Clinical
563 Practice Guidelines Expert Committee Pre-Existing Diabetes Preconception and
564 During Pregnancy. *Can J Diabetes* **42**, S255-282.
- 565 Foot N. J., and Kumar S. (2021). The Role of Extracellular Vesicles in Sperm Function
566 and Male Fertility. *Subcell. Biochem.* **97**, 483–500. doi:10.1007/978-3-030-67171-
567 6_19
- 568 Foster B. P., Balassa T., Benen T. D., Dominovic M., Elmadjian G. K., Florova V.,
569 Fransolet M. D., Kestlerova A., Kmiecik G., Kostadinova I. A., Kyvelidou C.,
570 Meggyes M., Mincheva M. N., Moro L., Pastuschek J., Spoldi V., Wandernoth P.,
571 Weber M., Toth B., and Markert U. R. (2016). Extracellular vesicles in blood, milk
572 and body fluids of the female and male urogenital tract and with special regard to
573 reproduction. *Crit. Rev. Clin. Lab. Sci.* **53**, 379–395.
574 doi:10.1080/10408363.2016.1190682
- 575 Galley J. D., and Besner G. E. (2020). The therapeutic potential of breast milk-derived

- 576 extracellular vesicles. *Nutrients* **12**, 745. doi:10.3390/nu12030745
- 577 Gardner D. K., and Balaban B. (2016). Assessment of human embryo development using
578 morphological criteria in an era of time-lapse, algorithms and ‘OMICS’: is looking
579 good still important? *Mol. Hum. Reprod.* **22**, 704–718. doi:10.1093/molehr/gaw057
- 580 Gillet V., Ouellet A., Stepanov Y., Rodosthenous R. S., Croft E. K., Brennan K.,
581 Abdelouahab N., Baccarelli A., and Takser L. (2019). MiRNA Profiles in
582 Extracellular Vesicles from Serum Early in Pregnancies Complicated by Gestational
583 Diabetes Mellitus. *J. Clin. Endocrinol. Metab.* **104**, 5157–5169.
584 doi:10.1210/jc.2018-02693
- 585 Giudice L. C. (2010). Endometriosis (DL Olive, Ed.). *N. Engl. J. Med.* **362**, 2389–2398.
586 doi:10.1056/NEJMcp1000274
- 587 Hromadnikova I., Dvorakova L., Kotlabova K., and Krofta L. (2019). The prediction of
588 gestational hypertension, preeclampsia and fetal growth restriction via the first
589 trimester screening of plasma exosomal C19MC microRNAs. *Int. J. Mol. Sci.* **20**,
590 doi:10.3390/ijms20122972
- 591 Hu J., Tang T., Zeng Z., Wu J., Tan X., and Yan J. (2020). The expression of small RNAs
592 in exosomes of follicular fluid altered in human polycystic ovarian syndrome. *PeerJ*
593 **2020**,. doi:10.7717/peerj.8640
- 594 Huang J., Zheng L., Wang F., Su Y., Kong H., and Xin H. (2020). Mangiferin ameliorates
595 placental oxidative stress and activates PI3K/Akt/mTOR pathway in mouse model
596 of preeclampsia. *Arch. Pharm. Res.* **43**, 233–241. doi:10.1007/s12272-020-01220-7
- 597 Huppertz B., Kadyrov M., and Kingdom J. C. P. (2006). Apoptosis and its role in the
598 trophoblast. *Am. J. Obstet. Gynecol.* **195**, 29–39. doi:10.1016/j.ajog.2005.07.039
- 599 Ji L., Xu R., Lu L., Zhang J., Yang G., Huang J., Wu C., and Zheng C. (2013). TM6, a
600 novel nuclear matrix attachment region, enhances its flanking gene expression

- 601 through influencing their chromatin structure. *Mol. Cells* **36**, 127–37.
602 doi:10.1007/s10059-013-0092-z
- 603 Johnstone R. ., Mathew A., Mason A. ., and Teng K. (1991). Exosome Formation During
604 Maturation of Mammalian and Avian Reticulocytes: Evidence That Exosome
605 Release Is a Major Route for Externalization. **2736**..
- 606 Khalaj K., Miller J. E., Lingegowda H., Fazleabas A. T., Young S. L., Lessey B. A., Koti
607 M., and Tayade C. (2019). Extracellular vesicles from endometriosis patients are
608 characterized by a unique miRNA-lncRNA signature. *JCI Insight* **4**.,
609 doi:10.1172/jci.insight.128846
- 610 Krysko D. V., Diez-Fraile A., Criel G., Svistunov A. A., Vandenabeele P., and D’Herde
611 K. (2008). Life and death of female gametes during oogenesis and folliculogenesis.
612 *Apoptosis* **13**, 1065–1087. doi:10.1007/s10495-008-0238-1
- 613 Kuklina E. V., Ayala C., and Callaghan W. M. (2009). Hypertensive disorders and severe
614 obstetric morbidity in the united states. *Obstet. Gynecol.* **113**, 1299–1306.
615 doi:10.1097/AOG.0b013e3181a45b25
- 616 Leaver R. B. (2016). Male infertility: an overview of causes and treatment options. *Br. J.*
617 *Nurs.* **25**, 35–41.
- 618 Lee D. C., Romero R., Kim J. S., Tarca A. L., Montenegro D., Pineles B. L., Kim E., Lee
619 J., Kim S. Y., Draghici S., Mittal P., Kusanovic J. P., Chaiworapongsa T., Hassan S.
620 S., and Kim C. J. (2011). MiR-210 targets iron-sulfur cluster scaffold homologue in
621 human trophoblast cell lines: Siderosis of interstitial trophoblasts as a novel
622 pathology of preterm preeclampsia and small-for-gestational-age pregnancies. *Am.*
623 *J. Pathol.* **179**, 590–602. doi:10.1016/j.ajpath.2011.04.035
- 624 Li T., Greenblatt E. M., Shin M. E. J., Brown T. J., and Chan C. (2020). Cargo small non-
625 coding RNAs of extracellular vesicles isolated from uterine fluid associate with

- 626 endometrial receptivity and implantation success. *Fertil. Steril.* 1–9.
627 doi:10.1016/j.fertnstert.2020.10.046
- 628 Liberati A., Altman D. G., Tetzlaff J., Mulrow C., Gøtzsche P. C., Ioannidis J. P. A.,
629 Clarke M., Devereaux P. J., Kleijnen J., and Moher D. (2009). The PRISMA
630 Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That
631 Evaluate Health Care Interventions: Explanation and Elaboration. *PLoS Med.* **6**,
632 e1000100. doi:10.1371/journal.pmed.1000100
- 633 Machtinger R., Laurent L. C., and Baccarelli A. A. (2016). Extracellular vesicles: Roles
634 in gamete maturation, fertilization and embryo implantation. *Hum. Reprod. Update*
635 **22**, 182–193. doi:10.1093/humupd/dmv055
- 636 Machtinger R., Rodosthenous R. S., Adir M., Mansour A., Racowsky C., Baccarelli A.
637 A., and Hauser R. (2017). Extracellular microRNAs in follicular fluid and their
638 potential association with oocyte fertilization and embryo quality: an exploratory
639 study. *J. Assist. Reprod. Genet.* **34**, 525–533. doi:10.1007/s10815-017-0876-8
- 640 Martinez R. M., Baccarelli A. A., Liang L., Dioni L., Mansur A., Adir M., Bollati V.,
641 Racowsky C., Hauser R., and Machtinger R. (2019). Body mass index in relation to
642 extracellular vesicle–linked microRNAs in human follicular fluid. *Fertil. Steril.* **112**,
643 387–396.e3. doi:10.1016/j.fertnstert.2019.04.001
- 644 Martinez R. M., Liang L., Racowsky C., Dioni L., Mansur A., Adir M., Bollati V.,
645 Baccarelli A. A., Hauser R., and Machtinger R. (2018). Extracellular microRNAs
646 profile in human follicular fluid and IVF outcomes. *Sci. Rep.* **8**, 1–10.
647 doi:10.1038/s41598-018-35379-3
- 648 Mendelson C. R. (2009). Minireview: Fetal-maternal hormonal signaling in pregnancy
649 and labor. *Molecular Endocrinology* **23**, 947–954. doi:10.1210/me.2009-0016
- 650 Menon R., Debnath C., Lai A., Guanzon D., Bhatnagar S., Kshetrapal P. K., Sheller-

- 651 Miller S., and Salomon C. (2019). Circulating exosomal miRNA profile during term
652 and preterm birth pregnancies: A longitudinal study. *Endocrinology* **160**, 249–275.
653 doi:10.1210/en.2018-00836
- 654 Motawi T. M. k., Sabry D., Maurice N. W., and Rizk S. M. (2018). Role of mesenchymal
655 stem cells exosomes derived microRNAs; miR-136, miR-494 and miR-495 in pre-
656 eclampsia diagnosis and evaluation. *Arch. Biochem. Biophys.* **659**, 13–21.
657 doi:10.1016/j.abb.2018.09.023
- 658 Nair S., Jayabalan N., Guanzon D., Palma C., Scholz-Romero K., Elfeky O., Zuñiga F.,
659 Ormazabal V., Diaz E., Rice G. E., Duncombe G., Jansson T., McIntyre H. D.,
660 Lappas M., and Salomon C. (2018). Human placental exosomes in gestational
661 diabetes mellitus carry a specific set of miRNAs associated with skeletal muscle
662 insulin sensitivity. *Clin. Sci.* **132**, 2451–2467. doi:10.1042/CS20180487
- 663 Ospina-Prieto S., Chaiwangyen W., Herrmann J., Groten T., Schleussner E., Markert U.
664 R., and Morales-Prieto D. M. (2016). MicroRNA-141 is upregulated in preeclamptic
665 placenta and regulates trophoblast invasion and intercellular communication.
666 *Transl. Res.* **172**, 61–72. doi:10.1016/j.trsl.2016.02.012
- 667 Pillay P., Vatish M., Duarte R., Moodley J., and Mackraj I. (2019). Exosomal microRNA
668 profiling in early and late onset preeclamptic pregnant women reflects
669 pathophysiology. *Int. J. Nanomedicine* **14**, 5637–5657. doi:10.2147/IJN.S208865
- 670 Pons-Rejraji H., Artonne C., Sion B., Brugnion F., Canis M., Janny L., and Grizard G.
671 (2011). Prostatosomes: Inhibitors of capacitation and modulators of cellular signalling
672 in human sperm. *Int. J. Androl.* **34**, 568–580. doi:10.1111/j.1365-
673 2605.2010.01116.x
- 674 Rodosthenous R. S., Burriss H. H., Sanders A. P., Just A. C., Dereix A. E., Svensson K.,
675 Solano M., Téllez-Rojo M. M., Wright R. O., and Baccarelli A. A. (2017). Second

- 676 trimester extracellular microRNAs in maternal blood and fetal growth: An
677 exploratory study. *Epigenetics* **12**, 804–810. doi:10.1080/15592294.2017.1358345
- 678 Ronquist G. (2012). Protasomes are mediators of intercellular communication: from
679 basic research to clinical implications. *J. Intern. Med.* **271**, 400–13.
680 doi:10.1111/j.1365-2796.2011.02487.x
- 681 Rooda I., Hasan M. M., Roos K., Viil J., Andronowska A., Smolander O.-P., Jaakma Ü.,
682 Salumets A., Fazeli A., and Velthut-Meikas A. (2020). Cellular, Extracellular and
683 Extracellular Vesicular miRNA Profiles of Pre-Ovulatory Follicles Indicate
684 Signaling Disturbances in Polycystic Ovaries. *Int. J. Mol. Sci.* **21**, 9550.
685 doi:10.3390/ijms21249550
- 686 Salas-Huetos A., James E. R., Aston K. I., Carrell D. T., Jenkins T. G., and Yeste M.
687 (2020). The role of miRNAs in male human reproduction: a systematic review.
688 *Andrology* **8**, 7–26. doi:10.1111/andr.12714
- 689 Salomon C., Guanzon D., Scholz-Romero K., Longo S., Correa P., Illanes S. E., and Rice
690 G. E. (2017). Placental exosomes as early biomarker of preeclampsia: Potential role
691 of exosomal microRNAs across gestation. *J. Clin. Endocrinol. Metab.* **102**, 3182–
692 3194. doi:10.1210/jc.2017-00672
- 693 Sandrim V. C., Luizon M. R., Palei A. C., Tanus-Santos J. E., and Cavallie R. C. (2016).
694 Circulating microRNA expression profiles in pre-eclampsia: Evidence of increased
695 miR-885-5p levels. *BJOG An Int. J. Obstet. Gynaecol.* **123**, 2120–2128.
696 doi:10.1111/1471-0528.13903
- 697 Sang Q., Yao Z., Wang H., Feng R., Wang H., Zhao X., Xing Q., Jin L., He L., Wu L.,
698 and Wang L. (2013). Identification of MicroRNAs in human follicular fluid:
699 Characterization of MicroRNAs that govern steroidogenesis in vitro and are
700 associated with polycystic ovary syndrome in vivo. *J. Clin. Endocrinol. Metab.* **98**,

- 701 3068–3079. doi:10.1210/jc.2013-1715
- 702 Simon C., Greening D. W., Bolumar D., Balaguer N., Salamonsen L. A., and Vilella F.
703 (2018). Extracellular vesicles in human reproduction in health and disease. *Endocr.*
704 *Rev.* **39**, 292–332. doi:10.1210/er.2017-00229
- 705 Sullivan R. (2016). Epididymosomes: Role of extracellular microvesicles in sperm
706 maturation. *Front. Biosci. (Schol. Ed)*. **8**, 106–114. doi:10.2741/S450
- 707 Sullivan R., and Saez F. (2013). Epididymosomes, prostasomes, and liposomes: their
708 roles in mammalian male reproductive physiology. *REPRODUCTION* **146**, R21–
709 R35. doi:10.1530/REP-13-0058
- 710 Sullivan R., Saez F., Girouard J., and Frenette G. (2005). Role of exosomes in sperm
711 maturation during the transit along the male reproductive tract. *Blood Cells, Mol.*
712 *Dis.* **35**, 1–10. doi:10.1016/j.bcmed.2005.03.005
- 713 Trams E. G., Lauter C. J., Salem N., and Heine U. (1981). Exfoliation of membrane ecto-
714 enzymes in the form of micro-vesicles. *Biochim. Biophys. Acta* **645**, 63–70.
715 doi:10.1016/0005-2736(81)90512-5
- 716 Truong G., Guanzon D., Kinhal V., Elfeky O., Lai A., Longo S., Nuzhat Z., Palma C.,
717 Scholz-Romero K., Menon R., Mol B. W., Rice G. E., and Salomon C. (2017).
718 Oxygen tension regulates the miRNA profile and bioactivity of exosomes released
719 from extravillous trophoblast cells-Liquid biopsies for monitoring complications of
720 pregnancy. *PLoS One* **12**, 1–27. doi:10.1371/journal.pone.0174514
- 721 Valadi H., Ekström K., Bossios A., Sjöstrand M., Lee J. J., and Lötvall J. O. (2007).
722 Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of
723 genetic exchange between cells. *Nat. Cell Biol.* **9**, 654–659. doi:10.1038/ncb1596
- 724 Vojtech L., Woo S., Hughes S., Levy C., Ballweber L., Sauteraud R. P., Strobl J.,
725 Westerberg K., Gottardo R., Tewari M., and Hladik F. (2014). Exosomes in human

- 726 semen carry a distinctive repertoire of small non-coding RNAs with potential
727 regulatory functions. *Nucleic Acids Res.* **42**, 7290–7304. doi:10.1093/nar/gku347
- 728 Vyas P., Balakier H., and Librach C. L. (2019). Ultrastructural identification of CD9
729 positive extracellular vesicles released from human embryos and transported
730 through the zona pellucida. *Syst. Biol. Reprod. Med.* **65**, 273–280.
731 doi:10.1080/19396368.2019.1619858
- 732 Wang Y., Du X., and Wang J. (2020). Transfer of miR-15a-5p by placental exosomes
733 promotes pre-eclampsia progression by regulating PI3K/AKT signaling pathway via
734 CDK1. *Mol. Immunol.* **128**, 277–286. doi:10.1016/j.molimm.2020.10.019
- 735 Wu L., Ding Y., Han S., and Wang Y. (2020). Role of Exosomes in the Exchange of
736 Spermatozoa after Leaving the Seminiferous Tubule: A Review. *Curr. Drug Metab.*
737 **21**, 330–338. doi:10.2174/1389200221666200520091511
- 738 Xu L., Wu L.-F., and Deng F.-Y. (2019). Exosome: An Emerging Source of Biomarkers
739 for Human Diseases. *Curr. Mol. Med.* **19**, 387–394.
740 doi:10.2174/1566524019666190429144310
- 741 Xueya Z., Yamei L., Sha C., Dan C., Hong S., Xingyu Y., and Weiwei C. (2020).
742 Exosomal encapsulation of miR-125a-5p inhibited trophoblast cell migration and
743 proliferation by regulating the expression of VEGFA in preeclampsia. *Biochem.*
744 *Biophys. Res. Commun.* **525**, 646–653. doi:10.1016/j.bbrc.2020.02.137
- 745 Yadava S. M., Feng A., Parobchak N., Wang B., and Rosen T. (2021). miR-15b-5p
746 promotes expression of proinflammatory cytokines in human placenta by inhibiting
747 Apelin signaling pathway. *Placenta* **104**, 8–15. doi:10.1016/j.placenta.2020.11.002
- 748 Zhang D., Lv J., Tang R., Feng Y., Zhao Y., Fei X., Chian R., and Xie Q. (2021).
749 Association of exosomal microRNAs in human ovarian follicular fluid with oocyte
750 quality. *Biochem. Biophys. Res. Commun.* **534**, 468–473.

751 doi:10.1016/j.bbrc.2020.11.058

752 Zhang W., Zhou X., Zhang H., Yao Q., Liu Y., and Dong Z. (2016). Extracellular vesicles

753 in diagnosis and therapy of kidney diseases. *American Journal of Physiology - Renal*

754 *Physiology* **311**, F844–F851. doi:10.1152/ajprenal.00429.2016

755 Zomer A., Vendrig T., Hopmans E. S., van Eijndhoven M., Middeldorp J. M., and Pegtel

756 D. M. (2010). Exosomes. *Commun. Integr. Biol.* **3**, 447–450.

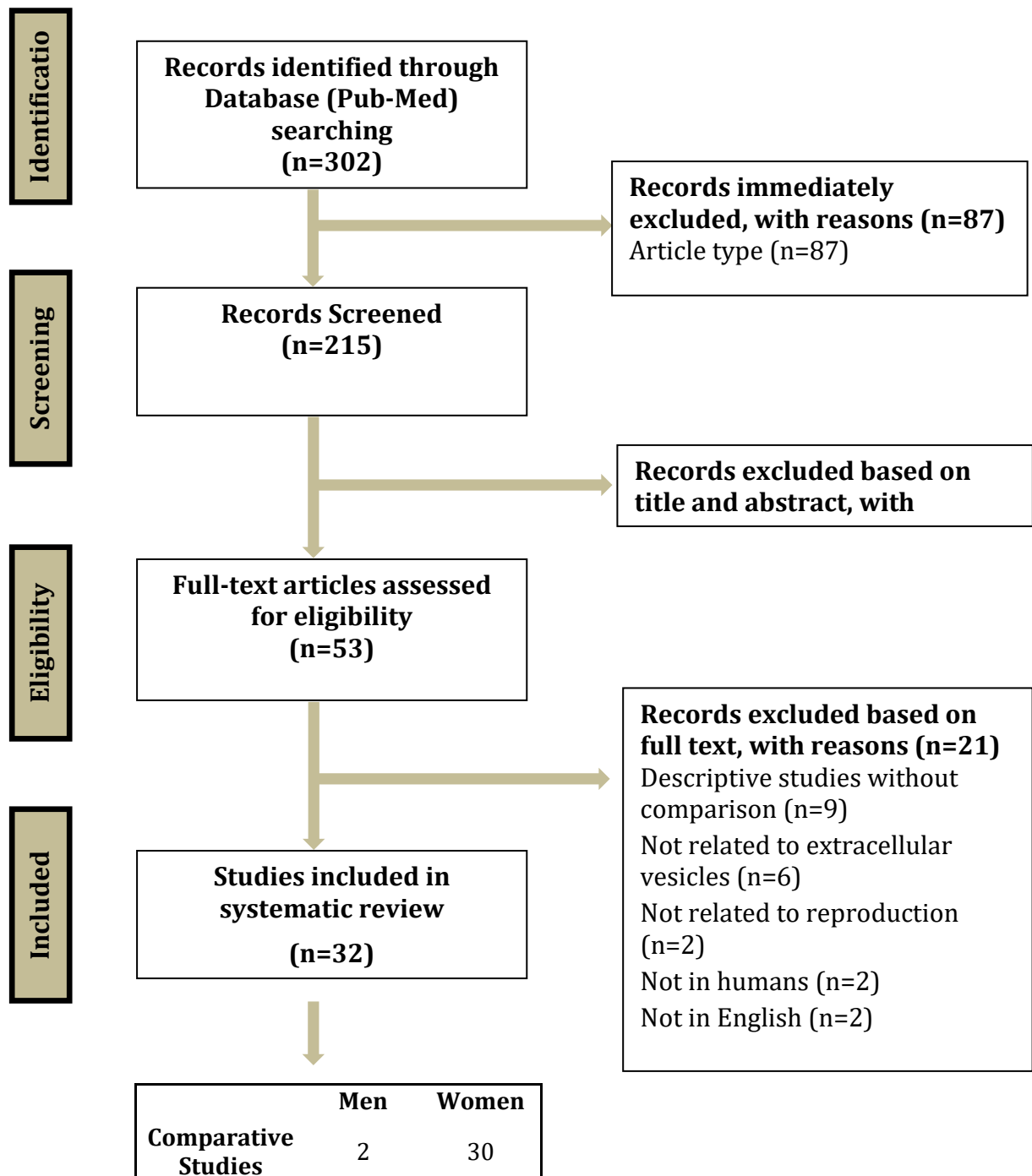
757 doi:10.4161/cib.3.5.12339

758

759 **FIGURE CAPTIONS**760 **Fig 1.** Flowchart of the literature search and selection process.

761

762



763 Table 1. Population, Intervention, Comparator, Outcome and Study (PICOS) design, with the inclusion
 764 and exclusion criteria and the keywords used for the definition of the search strategy and the eligibility of
 765 the study.

Parameter	Inclusion	Exclusion	Keywords
Population	Human (male and female)	Species other than humans	Human, <i>Homo sapiens</i>
Intervention	- miRNA identified after isolation and characterization of extracellular vesicles, and related to reproductive processes	- miRNA contained within extracellular vesicles not related to reproduction	miRNA, miRNA expression, exosome, extracellular vesicle, reproduction, fertility, embryo quality, ART, fertilization, implantation, infertility, oocyte, donor, sperm, maturation, differentiation, development, gamete, placenta, follicle, embryo culture, blastocyst, zona pellucida, follicular fluid
Comparison	- Expression of miRNAs encapsulated within extracellular vesicles related to reproductive disorders - Differential miRNA expression between fertile and infertile women - Differential miRNA expression between embryos of different quality - Differential miRNA expression between normal and abnormal pregnancies - Differential miRNA expression between fertile and infertile men	- Studies that do not study the miRNAs transported by extracellular vesicles and its association with human reproduction - Descriptive studies analyzing the miRNA content in a single population, but without comparison.	
Outcomes	- Fertility and assisted reproduction outcomes - miRNA dysregulation in reproductive issues - miRNAs as biomarkers for embryo quality		miRNA, expression profile, regulation, reproductive processes, pregnancy, ART outcome
Study design	- Research Article - Meta-analyses - Observational Study - Cross-sectional - Comparative - Longitudinal study	- Review article - Systematic reviews - Letters - Commentary articles	Research study, Comparative Study, Corrected and Republished Article, English Abstract, Journal Article, Observational Study, English, longitudinal study, cross-sectional study.

766

767

768 **Table 2.** Summary of the main identified microRNAs (miRNAs) encapsulated in extracellular vesicles (EVs) extracted from the comparative studies included in the systematic
769 review

770 **Table 2.** Summary of the main identified microRNAs (miRNAs) encapsulated in extracellular vesicles (EVs) extracted from the comparative studies included in the systematic
771 review

Reference	Objective of the study	Sex	Sample source	EVs isolation procedure	Comparison	Main miRNAs encapsulated in EVs up-/down-regulated		Results of the study/Main conclusion
						Upregulated	Downregulated	
(Barceló <i>et al.</i> 2018)	To determine whether the miRNA cargo of EVs from seminal plasma can be used as biomarkers to assess the origin of azoospermia and the presence of sperm in the testis	Male	Seminal plasma	Differential ultra-centrifugation	Azoospermic men VS normozoospermic men (control)	<p><i>Upregulated</i></p> hsa-miR-363-3p hsa-miR-365a-3p hsa-miR-29a-3p hsa-miR-296-5p hsa-miR-23b-5p hsa-miR-21-3p hsa-miR-193a-3p hsa-miR-29c-3p hsa-miR-361-3p hsa-miR-550a-5p hsa-miR-423-5p hsa-let-7f-1-3p hsa-miR-153-3p hsa-miR-196b-3p hsa-miR-96-5p	<p><i>Downregulated</i></p> hsa-miR-202-3p hsa-miR-514a-3p hsa-miR-202-5p hsa-miR-509-3-5p hsa-miR-510-5p hsa-miR-513c-5p hsa-miR-518e-3p hsa-miR-508-5p hsa-miR-520 hsa-miR-9-3p hsa-miR-506-3p hsa-miR-383-5p hsa-miR-34c-5p hsa-miR-517c-3p hsa-miR-873-5p hsa-miR-34b-5p hsa-miR-513a-3p hsa-miR-5211 hsa-miR-452-5p hsa-miR-122-5p hsa-miR-449a hsa-miR-499a-5p hsa-miR-455-5p hsa-miR-891b hsa-miR-890 hsa-miR-34c-3p hsa-miR-891a-5p hsa-miR-888-5p hsa-miR-124-3p hsa-miR-892a hsa-miR-551b-3p hsa-miR-424-5p	The study validated the potential of several miRNAs contained in EVs of seminal plasma as sensitive and specific biomarkers for selecting azoospermic individuals with real chances of obtaining spermatozoa from the testicular biopsy.

							hsa-miR-181b-5p hsa-miR-31-3p hsa-miR-181a-5p hsa-miR-31-5p hsa-miR-10b-3p hsa-miR-222-3p hsa-miR-455-3p hsa-miR-205-5p hsa-miR-182-3p hsa-miR-95-3p hsa-miR-9-5p hsa-miR-132-5p hsa-miR-203a		
(Abu-Halima et al., 2016)	To determine whether miRNA expression profile is different in EVs collected from seminal plasma of men with oligoasthenozoospermia to understand the underlying mechanisms of male infertility	Male	Seminal plasma	Differential ultra-centrifugation	Oligoasthenozoospermic subfertile men VS normozoospermic men (control)	Upregulated miR-1275 miR-4298 miR-3675-3p miR-765 miR-483-5p miR-1299 miR-766	Downregulated miR-4306 miR-28-5p miR-4286 miR-96 miR-185 miR-425 miR-100 miR-30e miR-331-3p miR-374a miR-15b miR-193b miR-30c miR-25 miR-27a miR-23a miR-27b miR-15a miR-93 miR-374b miR-200b miR-23b miR-20a miR-21 miR-148a miR-17 miR-30b miR-363 miR-26b	The study demonstrated an altered miRNA expression profile of EVs in seminal plasma from oligoasthenozoospermic subfertile men compared to normozoospermic fertile men.	
(Khalaj et al. 2019)	To determine the miRNA and proteomic content in EVs isolated from plasma and endometrial tissue of	Female	Endometrial tissue and blood plasma	Differential ultra-centrifugation	Women with endometriotic tissue VS women with normal endometrial tissue (control)	Upregulated hsa-miR-206 hsa-miR-29c-3p	Downregulated hsa-miR-1266-5p hsa-miR-200c-3p	The study demonstrated a miRNA signature contained within EVs	

patients with endometriosis (EMT) compared to patients with normal endometrial tissue, figuring out the potential role of these miRNAs in EVs on endometriosis pathophysiology

hsa-miR-139-3p
 hsa-let-7a-3p
 hsa-miR-95-3p
 hsa-miR-29b-3p
 hsa-miR-495-3p
 hsa-miR-136-3p
 hsa-miR-887-3p
 hsa-miR-381-3p
 hsa-miR-100-5p
 hsa-miR-193b-3p
 hsa-miR-335-5p
 hsa-miR-411-5p
 hsa-miR-451a
 hsa-miR-144-5p
 hsa-miR-486-5p

hsa-miR-200a-3p
 hsa-miR-20b-5p
 hsa-miR-200a-5p
 hsa-miR-96-5p
 hsa-miR-375
 hsa-miR-30d-5p
 hsa-miR-27a-3p

isolated from endometrial tissue from patients with endometriotic tissue by an up/down regulation of miRNAs. The miRNAs encapsulated in EVs were related to this pathology, and they were associated to an increasing of endothelial angiogenesis with a high increase in cellular growth.

(Chen <i>et al.</i> 2019)	To test whether myeloid-derived suppressor cells play a role in the progression of EMT, and to define EVs-miRNA profile in peritoneal fluid from endometriosis patients	Female	Peritoneal fluid	Differential ultra-centrifugation	Women with pregnancies complicated by endometriosis VS women with normal pregnancies (control)	Upregulated miR-1908-5p miR-130b miR-451a miR-486-5p miR-4488 miR-432-5p miR-342-5p miR-425-5p miR-505-5p	Downregulated miR-6508-3p miR-145-5p miR-365a-3p miR-365b-3p	The study reported that several EVs-miRNA were differentially expressed in the peritoneal fluid between endometriosis and healthy women and that these EVs-miRNAs were likely to be involved in the progression of endometriosis.
(Battaglia <i>et al.</i> 2020)	To identify the most significant dysregulated miRNAs contained in EVs in reproductive aging	Female	Follicular fluid	Differential ultra-centrifugation	Old (>38) VS young (<35) women subjected to <i>in vitro</i> Fertilization (IVF)	Upregulated miR-125b miR-155-5p miR-372	Downregulated miR-16-5p miR-214-3p miR-449a	The study proposed that different miRNAs carried by EVs isolated from follicular fluid could be responsible for some of the alterations detected in reproductive aging
(Diez-Fraile <i>et al.</i> 2014)	To report the presence of EVs-miRNAs in follicular fluid and to identify a set of miRNAs that are differentially expressed in older women compared to that of younger women	Female	Follicular fluid	Differential ultra-centrifugation	Old (>38) VS young (<31) women undergone to assisted reproduction	Upregulated old (>38) VS young (<31) women hsa-miR-134	-- hsa-miR-21-5p (only in young)	The study described the miRNA levels contained in EVs of follicular fluid together with a set of EVs-miRNAs differentially expressed in follicular fluid from young women and older women

						hsa-miR-190b and hsa-miR-99b-3p (only in old)		
(Hu <i>et al.</i> 2020)	To explore the role of miRNAs-containing EVs of follicular fluid in polycystic ovarian syndrome patients, in order to assess whether they can be used as potential biomarkers to early detect polycystic ovarian syndrome	Female	Follicular fluid	Differential ultra-centrifugation	Women with pregnancies complicated by polycystic ovarian syndrome VS women with normal pregnancies (control)	Upregulated miR-6087 miR-4745-3p miR-193b-3p miR-199a-5p miR-4532 miR-199a-3p miR-199b-3p miR-629-5p miR-143-3p miR-25-3p	Downregulated miR-98-5p miR-483-5p miR-382-5p miR-23b-3p miR-10a-5p miR-200a-3p miR-141-3p miR-3911 miR-200c-3p miR-483-3p	The study found that the expression of several miRNAs-EVs of follicular fluid differed between polycystic ovarian syndrome and non-polycystic ovarian syndrome patients. The miRNAs contained in EVs may play a key role in the mechanism that leads polycystic ovarian syndrome pathogenesis, and can act as biomarkers for polycystic ovarian syndrome diagnosis
(Rooda <i>et al.</i> 2020)	To investigate the difference in the miRNA profile contained in EVs of follicular fluid from normal women and polycystic ovarian syndrome patients.	Female	Follicular fluid	Size exclusion chromatography (SEC)	Women with pregnancies complicated by polycystic ovarian syndrome VS women with normal pregnancies (control)	Upregulated hsa-miR-200c-3p hsa-miR-100-5p hsa-miR-10a-5p hsa-miR-342-3p hsa-miR-28-3p hsa-miR-125b-5p	Downregulated hsa-miR-17-5p	The study evidenced that polycystic ovarian syndrome patients had alterations in the miRNA expression profile in EVs isolated from follicular fluid that can lead to changes in estrogen receptor signaling, apoptosis and the dysregulation of transcription affecting the progression of the disease
(Sang <i>et al.</i> 2013)	To identify EVs-miRNAs in follicular fluid and to investigate the role they play in polycystic ovarian syndrome	Female	Follicular fluid	Differential ultra-centrifugation	Women with pregnancies complicated by polycystic ovarian syndrome VS women with normal pregnancies (control)	Upregulated	Downregulated miR-132 miR-320	The study demonstrated that there are several miRNAs in follicular fluid some of them play a key roles in steroidogenesis and polycystic ovarian syndrome
(Martinez <i>et al.</i> 2018)	To assess whether EV-miRNAs from follicular fluid can serve as biomarkers for fertilization status and day 3 embryo quality	Female	Follicular fluid	Differential ultra-centrifugation	Fertilization status: failed to fertilize VS Normally fertilized Day 3 Embryo quality: poor quality embryo VS high quality embryo	Upregulated Fertilization status hsa-miR-92a hsa-miR-130b Poor VS high quality hsa-miR-888	Downregulated -- hsa-miR-214	The study suggested that EV-miRNAs of follicular fluid may play a role in pathways of ovarian function and follicle development, which could be essential for understanding the

						hsa-miR-454	molecular mechanisms that could lead to a successful pregnancy and birth	
(Zhang <i>et al.</i> 2021)	To investigate EVs-microRNAs in follicular fluid and explore their potential association with oocyte quality.	Female	Follicular fluid	Differential ultra-centrifugation	Poor oocyte quality VS High oocyte quality	Upregulated hsa-miR-1246 hsa-miR-548ae-5p hsa-miR-505-3p hsa-miR-548t-3p hsa-miR-548au-5p hsa-miR-320e hsa-miR-1303	Downregulated hsa-miR-513c-5p hsa-miR-548au-3p	The study indicated that the dysregulated miRNAs contained within EVs isolated from follicular fluid may be potential biomarkers for evaluating oocyte quality.
(Machtinger <i>et al.</i> 2017)	To determine the profile of miRNAs contained within EVs isolated from follicular fluid and explore their association with fertilization potential and embryo quality.	Female	Follicular fluid	Commercial kit (exoRNeasy kit [Qiagen])	Fertilization status: failed to fertilize VS fertilized Day 3 Embryo quality: poor quality embryo VS high quality embryo	Upregulated Not fertilized VS normally fertilized -- Poor VS high quality	Downregulated miR-202-5p miR-206 miR-16-1-3p miR-1244 miR-663b miR-766-3p miR-132-3p hsa-miR-16-5p	The study suggested that miRNAs contained in EVs of follicular fluid can lead to downstream events that will affect fertilization and day 3 embryo quality and morphology.
(Li <i>et al.</i> 2020)	To characterize EVs-miRNAs from uterine fluid, aimed to uncover endometrial receptivity-associated biomarkers	Female	Uterine Fluid	Differential ultra-centrifugation	Women with pregnancies aided by controlled ovarian stimulation VS women with normal pregnancies (control)	Upregulated hsa-miR-362-3p	Downregulated --	The study identified a differential expression of miR-362-3p in EVs isolated from uterine fluid in patients who conceived compared to those who did not. This miRNA seems to be associated with biological functions related to immune response, extracellular matrix, and cell junction.
(Hromadnikova <i>et al.</i> 2019)	To evaluate whether placental C19MC miRNAs in plasma EVs would be able to predict, during the early stages of gestation, patients that will develop pregnancy-related complications and	Female	Blood plasma	Commercial kit (miRCURY™ Exosome Isolation Kit-[Exiqon])	Women with pregnancies complicated by preeclampsia and/or fetal growth restriction VS women with normal pregnancies (control)	Upregulated	Downregulated miR-517-5p	This study indicated that the miRNAs contained within EVs released to the systemic circulation by the placenta may be used as a

women that will have normal progression of gestation

miR-520a-5p
miR-525-5p

part of first trimester pregnancy screening to identify women with risk to develop a pregnancy-related complication such as preeclampsia and fetal growth restriction

(Salomon <i>et al.</i> 2017)	To investigate whether EVs and their miRNA cargo present in blood plasma of pregnant women can be used as early biomarker for preeclampsia.	Female	Blood plasma	Commercial kit (miRNeasy Mini Kit [Qiagen])	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control)	Upregulated hsa-miR-486-1-5p hsa-miR-486-2-5p hsa-miR-423-5p hsa-miR-451a hsa-miR-107 hsa-miR-15a-5p hsa-miR-335-5p has-miR-92a-2-3p hsa-miR-103-1-3p hsa-miR-103-2-3p has-miR-92a-1-3p	Downregulated hsa-miR-126-3p	This study evidenced that the evaluation of the miRNAs carried by EVs isolated from blood plasma of pregnant women could have a diagnostic value for predict women with risk for developing preeclampsia. This study pointed out hsa-miR-486-1-5p and hsa-miR-486-2-5 as potential biomarkers that can be used to differentiate between normal and preeclampsia pregnancies.
(Xueya <i>et al.</i> 2020)	To examine the association between hsa-miR-125a-5p within EVs isolated from umbilical cord blood with preeclampsia.	Female	Umbilical cord blood	Commercial kit (exoRNeasy Serum/Plasma Kit [Qiagen])	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control)	Upregulated miR-125a-5p	Downregulated	The study assessed that miR-125a-5p expression in EVs isolated from umbilical cord blood in preeclampsia patients was higher than in normal patients. It was demonstrated that dysregulation of miR-125a-5p in EVs might affect HTR8/SVneo cell proliferation and migration and inhibit angiogenesis, indicating that miR-125a-5p was involved in the progression of preeclampsia
(Biró <i>et al.</i> 2019)	To investigate whether and the miRNAs EVs isolated from blood plasma in pregnant women can be used as early biomarkers for preeclampsia	Female	Blood plasma and placenta samples	Commercial kit (ExoRNeasy kit, [Qiagen])	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control)	Upregulated hsa-miR-210	Downregulated	The study postulated that in preeclampsia, the hsa-miR-210 contained in EVs is secreted dynamically from the trophoblast, and it may have a key role in the etiology of this disease

(Pillay <i>et al.</i> 2019)	To better understand the pathophysiological role of miRNAs of EVs isolated from blood plasma in preeclampsia process (in early and late onset preeclampsia)	Female	Blood plasma	Commercial kit (miRCURY Exosome isolation kit [Qiagen])	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control)	<i>Upregulated</i>	<i>Downregulated</i>	This study identified EVs-miRNAs signatures in early onset preeclampsia and late onset preeclampsia involved in the regulation of preeclampsia associated processes
						<i>Early onset Preeclampsia VS Control</i>		
						hsa-miR-223-3p	hsa-miR-431-5p	
						hsa-miR-490-3p	hsa-miR-758-5p	
						hsa-miR-874-3p		
						hsa-miR-126-3p		
						hsa-miR-190a-5p		
						hsa-miR-23a-3p		
						hsa-miR-324-3p		
						<i>Late onset Preeclampsia VS Control</i>		
						hsa-miR-297	hsa-miR-375	
						hsa-miR-202-3p	hsa-miR-488-3p	
						hsa-miR-499a-5p	hsa-miR-505-3p	
						hsa-miR-640	hsa-miR-296-3p	
(Wang <i>et al.</i> 2020)	To investigate the role of placental derived EVs and their miRNA cargo, (miR-15a-5p) in preeclampsia	Female	Blood plasma	Differential ultra-centrifugation	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control)	<i>Upregulated</i>	<i>Downregulated</i>	The study provided evidence that transfer of miR-15a-5p by placental EVs could be a promising therapeutic target to prevent preeclampsia
						miR-15a-5p	--	
(Truong <i>et al.</i> 2017)	To investigate whether oxygen tension is able to modify the EVs release and miRNA profile from extravillous trophoblast cells, altering their bioactivity on endothelial cells. This study also aimed to establish the EVs-miRNA profile at early gestation in women who will develop preeclampsia and spontaneous preterm birth	Female	Blood plasma	Differential ultra-centrifugation	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control) Women with preterm birth delivered VS women with term birth delivered (control)	<i>Upregulated</i>	<i>Downregulated</i>	The study demonstrated that low oxygen tension caused by pregnancy-related complications promote the release of EVs from extravillous trophoblast cells. The miRNAs of EVs were able to modify the migration capacity and release of TNF α from endothelial cells, which seems to be related to preeclampsia and preterm birth pathophysiology
						<i>Preeclampsia VS with normal pregnancies</i>		
						miR-744-5p	miR-335-5p	
						miR-584-5p	miR-192-5p	
						let-7a-5p	miR-23a-3p	
						miR-6724-5p	miR-144-3p	
						miR-17-5p	miR-125b-2-3p	
						miR-199a-3p	miR-542-3p	
						miR-141-3p	miR-205-5p	
						miR-30c-5p	miR-208a-3p	
						miR-26a-5p	miR-518a-3p	
						miR-221-3p	miR-451a	
						<i>Preterm birth compared with normal pregnancies</i>		

						let-7a-5p miR-17-5p miR-92a-3p miR-191-5p miR-151-3p miR-423-5p miR-344d-3p miR-32-3p	miR-145-3p miR-4792 miR-344a-5p miR-889-3p miR-625-5p	
(Biró <i>et al.</i> 2017)	To measure total EVs-miRNA concentration and to perform expression analysis of circulating EVs miRNA hsa-miR-210 in women affected by chronic hypertension or gestational hypertension or preeclampsia	Female	Blood plasma	Commercial kit (Exosome precipitation solution [Macherey-Nagel GmbH])	Women with pregnancies complicated by preeclampsia, chronic hypertension or gestational hypertension VS women with normal pregnancies (control)	Upregulated hsa-miR-210	Downregulated	The study stated that the concentration of total circulating EVs-miRNA and the levels of hsa-miR-210 were higher in blood samples of pregnant women with preeclampsia. It was demonstrated that hsa-miR-210 was secreted via EVs and that it could have a key role in the pathogenicity of the disease
(Sandrim <i>et al.</i> 2016)	To validate and to compare the miRNA expression profiles of EVs isolated from blood plasma between pregnant women with preeclampsia and those with normal pregnancy	Female	Blood plasma	Commercial kit (miRNeasy Kit [Qiagen])	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control)	Upregulated miR-885-5p	Downregulated miR-376c-3p miR-19a-3p miR-19b-3p	The study demonstrated that miR-885-5p transported by EVs was increased in blood plasma from preeclampsia patients compared with healthy pregnant women, which can be considered as a putative biomarker of this pathology
(Motawi <i>et al.</i> 2018)	To evaluate the expression of miR-136, miR-494 and miR-495 in EVs isolated from of blood plasma and uterine cord blood as putative biomarkers for preeclampsia.	Female	Blood plasma and Umbilical cord blood	Differential ultra-centrifugation	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control)	Upregulated miR-136 miR-494 miR-495	Downregulated	The study suggested that miRNA-136, miRNA-494 and miRNA-495 transported by EVs could be promising circulating biomarkers in early detection of preeclampsia
(Cronqvist <i>et al.</i> 2017)	To investigate the uptake of placenta derived-EVs by primary coronary artery endothelial cells in women with normal pregnancy and preeclampsia	Female	Placental cotyledons	Differential ultra-centrifugation	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control)	Upregulated miR-517a miR-517c miR-519a	Downregulated	The study revealed an internalization of placenta derived-EVs into primary coronary artery endothelial cells, and a transfer of placenta specific miRNAs into the endoplasmic reticulum

and mitochondria of these recipient cells. Further, the miRNAs contained by EVs led to a down regulation of specific preeclampsia associated target genes.

(Ospina-Prieto <i>et al.</i> 2016)	To determine whether miR-141 carried in EVs is differently expressed between placental tissues of women with preeclampsia VS healthy women	Female	Human Placental Trophoblasts (PHT)	Differential ultra-centrifugation	Women with pregnancies complicated by preeclampsia VS women with normal pregnancies (control)	Upregulated miR-141	Downregulated	The study demonstrated that the expression of miR-141 contained in EVs of PHT was higher in preeclampsia patients compared with those from normal pregnancies
(Menon <i>et al.</i> 2019)	To characterize serial changes in the miRNA content in EVs present in maternal blood plasma across gestation in term and preterm birth pregnancies, in order to find potential biomarkers that could predict preterm birth	Female	Blood plasma	Differential ultra-centrifugation	Women with preterm birth delivered VS women with term birth delivered (control)	Upregulated hsa-miR-145-5p hsa-let-7b-3p hsa-miR-197-3p hsa-miR-10a-3p hsa-miR-145-5p hsa-miR-128-1-3p hsa-miR-202-5p hsa-miR-1275	Downregulated hsa-miR-148a-3p hsa-miR-1304-3p hsa-miR-101-1-3p hsa-miR-1304-5p hsa-miR-1304-3p hsa-let-7i-3p hsa-miR-1249-5p hsa-miR-1255b-2-3p	The study demonstrated that circulating EVs in blood plasma of pregnant women carried a specific set of miRNAs that changed across the gestation, and that this miRNA profile in EVs differed between preterm birth pregnancies compared to normal term deliveries. Specifically, this study found that 173 miRNAs changed across gestation for normal compared with preterm birth pregnancies
(Fallen <i>et al.</i> 2018)	To report a comprehensive signature of miRNA carried by EVs isolated from blood plasma of pregnant women with preterm birth and to reveal the usefulness of EV-associated miRNAs in the diagnosis of this pathology	Female	Blood plasma	SEC	Women with preterm birth delivered VS women with term birth delivered (control)	Upregulated hsa-miR-192-5p hsa-miR-194-1-5p hsa-miR-378c-5p hsa-miR-4326-5p hsa-miR-505-5p hsa-miR-589-3p hsa-miR-671-5p hsa-mir-7641-2 hsa-miR-92a-2-3p hsa-miR-214-3p	Downregulated hsa-miR-100-5p hsa-miR-127-5p hsa-miR-136-3p hsa-miR-141-3p hsa-miR-337-3p hsa-miR-337-5p hsa-miR-33a-3p hsa-miR-369-3p hsa-miR-369-5p hsa-miR-376b-3p hsa-miR-376c-3p hsa-miR-377-3p	The study demonstrated an altered profile of EVs-miRNA in blood plasma from women with preterm birth compared to normal pregnancies. It was reported that EV-associated miRNA could be a useful and relatively non-invasive source of biomarkers for preterm birth

						hsa-miR-379-3p hsa-miR-379-5p hsa-miR-380-3p hsa-miR-382-3p hsa-miR-410-3p hsa-miR-411-5p hsa-miR-431-5p hsa-miR-487b-3p hsa-miR-495-3p hsa-miR-512-1-5p hsa-miR-515-1-3p hsa-miR-515-1-5p hsa-miR-516b-1-5p hsa-miR-517a-3p hsa-miR-517c-3p hsa-miR-518b-3p hsa-miR-518c-3p hsa-miR-518f-3p hsa-miR-519d-3p hsa-miR-520d-5p hsa-miR-524-5p hsa-miR-525-5p hsa-miR-526b-5p hsa-miR-539-3p hsa-miR-551b-3p hsa-miR-590-3p hsa-miR-655-3p hsa-miR-656-3p hsa-miR-889-3p		
(Yadava <i>et al.</i> 2021)	To investigate the role of miRNAs carried by fetal EVs in the regulation of placental gene expression and their involvement in preterm birth	Female	Fetal cord arterial blood	Differential ultra-centrifugation	Women with preterm birth delivered by cesarean VS women with term birth delivered (control)	<i>Upregulated</i> miR-6727-5p	<i>Downregulated</i> let-7i-5p miR-185-5p miR-548d-5p miR-92b-3p miR-16-5p miR-1301-3p	The study found that miR-15b-5p carried by placental EVs can activate pro-labor hormones and cytokines including IL-1, IL-6, IL-8, and TNF- α .

(Gillet <i>et al.</i> 2019)	To compare the miRNAs expression in EVs isolated from blood plasma of women with pregnancies complicated by gestational diabetes mellitus compared to women with normal pregnancies	Female	Blood plasma	Differential ultra-centrifugation	Women with pregnancies complicated by gestational diabetes VS women with normal pregnancies (control)	<i>Upregulated</i>	miR-15b-5p miR-376c-3p <i>Upregulated</i> miR-122-5p miR-136-5p miR-29a-3p miR-132-3p miR-1323 miR-210-3p miR-520h miR-29b-3p miR-342-3p miR-182-3p	The results evidenced that miRNAs contained within EVs were involved in trophoblast proliferation as well as in insulin regulation and transport of glucose in pregnant women. The analysis of miRNAs-EVs isolated from blood plasma of pregnant women could be a promising tool for studying the early effect of impaired glucose metabolism on placental development
(Nair <i>et al.</i> 2018)	To investigate whether placental EVs from patients with gestational diabetes mellitus carry a specific set of miRNAs associated with skeletal muscle insulin sensitivity	Female	Chorionic villous explants	Differential ultra-centrifugation	Women with pregnancies complicated by gestational diabetes mellitus VS women with normal pregnancies (control)	<i>Upregulated</i>	hsa-miR-125a-3p hsa-miR-224-5p hsa-miR-584-5p hsa-miR-186-5p hsa-miR-22-3p hsa-miR-99b-5p hsa-miR-433-3p hsa-miR-197-3p hsa-miR-423-3p <i>Downregulated</i> hsa-miR-208a-3p hsa-miR-335-5p hsa-miR-451a hsa-miR-145-3p hsa-miR-369-3p hsa-miR-483-3p hsa-miR-203a-3b hsa-miR-574-3p hsa-miR-144-3p hsa-miR-6795-5p hsa-miR-550a-3-3p hsa-miR-411-5p hsa-miR-550a-3-3p has-miR-140-3p	This study found that the concentration of EVs was higher in women with gestational diabetes mellitus compared to normal glucose tolerant women. In addition, it was found a differential miRNA expression in EVs released from the chorionic villous explants of women with gestational diabetes mellitus compared to those from women with normal pregnancies. These differential miRNAs transported by EVs were related to insulin resistance and carbohydrates metabolism genes
(Martinez <i>et al.</i> 2019)	To study whether increased body mass index is associated with altered expression of miRNAs carried by EVs of follicular fluid	Female	Follicular fluid	Differential ultra-centrifugation	Women undergone in vitro fertilization (IVF) with different BMI.	<i>Upregulated</i>	hsa-miR-328 <i>Downregulated</i>	These results showed that a 1-unit increase in body mass index was associated with an altered miRNAs expression of hsa-miR-328 contained in EVs of follicular fluid that may influence follicular and

oocyte developmental pathways

(Rodosthenous <i>et al.</i> 2017)	To determine the association of EVs-miRNAs profile with abnormal fetal growth comparing mothers of infants classified as small-for-gestational age and large-for-gestational age to appropriate-for-gestational age, matched by gestational age at delivery.	Female	Blood plasma	Commercial kit (exoRNeasy kit [Qiagen])	Small and large fetal growth for gestational age compared with appropriate fetal growth	<i>Upregulated</i>	<i>Downregulated</i>	The study suggested that EVs-miRNAs circulating in blood plasma in pregnant women at second trimester were associated with fetal growth
						<i>Small fetal growth VS appropriate fetal growth</i>		
						--	miR-20b-5p	
							miR-942-5p	
							miR-324-3p	
							miR-223-5p	
							miR-127-3p	
						<i>Large fetal growth VS appropriate fetal growth</i>		
						miR-661	--	
						miR-197-3p		
						miR-212-3p		

772

773

774