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

https://doi.org/10.1071/RD21301

Title

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

Running title

Extracellular vesicles miRNA in human reproduction

Authors

Isabel Barranco[1,*], Albert Salas-Huetos[2,3,4], Angel Berlanga[2,3], Marcella Spinaci [1], Marc Yeste[2,3], Jordi Ribas-Maynou [2,3,*]

Affiliations

[1]Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy.

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

[3]Unit of Cell Biology, Department of Biology, Faculty of Sciences, University of Girona, Girona, Spain.

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

*Corresponding authors:

Dr. Jordi Ribas-Maynou.

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

Contact: jordi.ribasmaynou@udg.edu. Tel.: +34 972 419514; Fax: +34 972 418150. ORCiD: 0000-0002-9101-2044

Dr. Isabel Barranco.
Address: Department of Veterinary Medical Sciences, University of Bologna, IT-40064 Ozzano dell'Emilia, Bologna, Italy. +39 051 2097904. isabel.barranco@unibo.it. ORCiD: 0000-0001-9873-814X

Data availability statement

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

Funding

The authors acknowledge the support from the Ministry of Science and Innovation, Spain (Grant: IJC2019-039615-I, A.S-H.); the Regional Government of Catalonia, Spain (Grant: 2017-SGR-1229, M.Y.); European Union’s Horizon 2020 research and innovation programme (Grant: H2020-MSCA-IF-2019-891382, I.B.) and European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie agreement No. 801342 (Grant: TECSPR-19-1-0003, J.R.M.).

Conflicts of interest

Marc Yeste is an Editor of Reproduction, Fertility and Development, but was blinded from the peer review process for this paper.

Acknowledgements

Not applicable

Summary text

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

Abstract

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

Keywords: Extracellular vesicles, exosomes, microvesicles, microRNAs, reproduction, reproductive disorders,

Introduction

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

Human reproduction is a complex process involving a wide variety of cell types that require crosstalk to achieve an adequate regulation at molecular level in order to perform their function. The EVs are proven to be involved in reproductive processes at many levels, from gamete generation and maturation to embryo implantation, both in men and women (Sullivan 2016;; Simon *et al.* 2018; Vyas *et al.* 2019; Baskaran *et al.* 2020; Foot and Kumar 2021). Each reproductive tissue is known to release specific EVs, which have an unique cargo with a particular function in both the male and female genital tract (Machtinger *et al.* 2016; Andronico *et al.* 2019). Specifically, it has been reported that the miRNA cargo of EVs (EV miRNAs) is involved in key processes such as gamete maturation, embryo development, immune modulation and cell invasion (Sullivan *et al.* 2005; Bechoua *et al.* 2011; Pons-Rejraji *et al.* 2011; Vojtech *et al.* 2014). The transfer of miRNAs from donor to recipient cells through EVs has been previously demonstrated, thus conferring the ability of modifying their functions (Valadi *et al.* 2007). Previous studies also suggested that EV miRNAs can be used to determine the quality of oocytes or to help verify the positive or negative outcome of an *in vitro* fertilization (IVF) process, thus being a potential biomarker for the prediction of IVF outcomes in humans (Martinez *et al.* 2018). Finally, the identification of miRNA cargo in EVs has also been shown to anticipate the progression of some reproductive-related diseases, such as polycystic ovary syndrome (PCOS), preeclampsia or pre-term birth (Simon *et al.* 2018). While it is still unclear whether the dysregulation of this EV miRNA cargo could be the cause or the consequence of these disorders, future studies could uncover the potential roles of these EV miRNAs and help us to draw specific biomarkers or even treatments (Xu *et al.* 2019). In this systematic review, therefore, we will focus on the miRNA cargo of EVs related to human reproductive biology and the consequences/causes of their dysregulation. Thus, the objective is to comprehensively and systematically collect the updated data about the role of miRNA carried by EVs in reproductive physiology, identifying the miRNAs encapsulated in EV in different fluids that are related to pathological reproductive processes.

Materials and methods

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

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

Data sources and search strategy

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

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

Study selection and eligibility procedure

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

Selection of studies started once all records were annotated in the database; article types declared as non-eligible were directly excluded. The second stage in study selection was based on title and abstract screening, excluding those articles that did not meet the eligibility criteria. Thereafter, the full text of all selected articles was downloaded and screened for a third step of exclusion, that was conducted to obtain the final list of selected articles.

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

Data extraction for systematic review

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

Results

Identification and selection of the studies

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

Selected studies overview

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

Studies included had a comparative objective, i.e., subjects displaying abnormal/pathological reproductive condition vs. normal/health (Table 2). Out of the 32 studies included, two were focused in men and the other 30 investigated female-related reproductive disorders. The male-factor studies examined the expression profile of EV miRNA in seminal plasma, assessing the potential relationship of miRNAs encapsulated within EVs with oligoastenozoospermia/azoospermia. Among the studies focused in female factors, one examined the differential miRNA expression profile between EVs released from endometriotic and normal endometrial tissue; 15 examined the differential EV miRNA expression profile in blood plasma between healthy and pregnancy-related complications such as preterm birth (n = 3), gestational diabetes (n = 1), preeclampsia (n = 10) and fetal growth (n = 1); three examined the differential expression profile in placenta-derived EV miRNA between healthy and pregnancy-related complications, such as gestational diabetes (n =1), and preeclampsia (n = 2); nine examined the differential miRNA expression profile in follicular fluid derived EVs, three in normal and PCOS-pregnancies, two in patients with different age, one in patients with different body mass index and three in oocytes or pre-implantation embryos of different quality; one examined miRNAs in EVs isolated from uterine fluid in order to find receptivity associated biomarkers; and one article examined the differential miRNA expression profile of EV isolated from peritoneal fluid between endometriosis and healthy women.

Discussion

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

Role of miRNAs carried by EVs in male reproductive physiology

Because infertility due to the male factor affects half of infertile couples (Leaver 2016), new, non-invasive biomarkers are needed to predict the chances of having a successful pregnancy in these couples. Growing evidence points to seminal EVs as key modulators of sperm physiological processes, including sperm maturation, motility, capacitation, and acrosome reaction, influencing the fertilization process (Ronquist 2012; Sullivan and Saez 2013; Baskaran *et al.* 2020; Wu *et al.* 2020). Two studies included in this systematic review (Abu-Halima *et al.* 2016; Barceló *et al.* 2018) were focused on the analysis of the miRNAs contained in seminal plasma EVs and aimed at uncovering the causes and biomarkers of oligo/azoospermia. The assessment of more than 600 mature miRNAs in these two studies showed that several miRNAs were dysregulated in azoospermic men; specifically, 36 in Abu-Halima *et al*. (2016) and 60 in Barceló *et al*. (2018). Surprisingly, while four of these dysregulated miRNAs (miR-23b, miR-21, miR-363 and miR-96) were identified in both studies, they exhibited an opposite pattern. Differences in the RNA isolation method, miRNA analysis or patient selection between these two studies could contribute to explain these inconsistent results.

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

Role of miRNAs carried by EVs in female reproductive processes

Endometriosis

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

Reproductive aging

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

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

Polycystic ovarian syndrome (PCOS)

Polycystic ovarian syndrome usually courses with hyperandrogenism, obesity, polycystic ovarian morphology, insulin resistance and/or anovulation, thus affecting oocyte quality. Three studies included in this review (Sang *et al.* 2013; Hu *et al.* 2020; Rooda *et al.* 2020) compared the expression profile of the EV-miRNAs present in the follicular fluid between women suffering from PCOS and those not suffering from that disease. The three studies demonstrated that several miRNAs transported by EVs were involved in amino acid and glycosaminoglycan biosynthesis, and that carbon and monocarboxylic metabolism was dysregulated in PCOS patients (Sang *et al.* 2013; Hu *et al.* 2020; Rooda *et al.* 2020). In these three studies, the main over- and under-expressed miRNAs (Table 2) were proposed to be potential early biomarkers of this disorder; however, their utility remains controversial, as opposite results were found for two miRNA (miR-10a-5p and miR-200c-3p), which were down- (Hu et al., 2020) and upregulated (Rooda et al., 2020), respectively. In this regard, it can be hypothesized that differences could be due to the method used to isolate EVs (ultracentrifugation for Hu et al., 2020 *vs* chromatography for Rooda et al., 2020), but one has to take into account that other factors, such as the RNA isolation method, differed between these studies. Moreover, variables such as the use of different patient/donor cohorts may also explain such differences. For all these reasons, more research needs to be conducted to reduce these uncertainties, before accepting the clinical utility of these miRNAs.

Role of miRNAs carried by EVs in pregnancy-related processes

Embryo/Oocyte quality

While the success rates of single embryo transfer following ICSI in humans have been improved in the last decades, mounting evidence supports that they have reached a plateau (European IVF-monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE) *et al.*, 2020). Despite the usefulness of classical embryo parameters, many efforts are focused on uncovering potential biomarkers that could have better predictive ability upon embryo implantation and the achievement of life birth (Gardner and Balaban 2016). In this regard, three studies included in our review aimed at comparing the follicular fluid-derived EV miRNA cargoes between top- and poor-quality oocytes/preimplantation embryos (Machtinger *et al.* 2017; Martinez *et al.* 2018; Zhang *et al.* 2021). The identification of miRNAs encapsulated in EV led to the finding of several dysregulated miRNAs in the follicular fluid of oocytes that failed to be fertilized. The dysregulated miRNAs from embryos with fertilization failure reported in the studies (Table 2) were predicted to target genes involved in organ development, reproductive system diseases and systemic abnormalities. In the same way, miRNA dysregulation was identified in follicular fluid EVs isolated from follicles that led to poor-quality embryos. These miRNAs were found to be involved in follicular growth, regulation of oocyte meiosis, cellular signaling and ovarian function pathways (Martinez *et al.* 2018). All these findings suggest that follicular fluid EV-borne miRNAs could be crucial for proper embryo development and fertilization, and could be used as potential biomarkers to predict embryo quality and pregnancy success.

Preeclampsia

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

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

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

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

Preterm birth

While the initiation of parturition occurs when fetal development is completed and is related to immune and feto-maternal endocrine changes in the uterine cavity (Mendelson 2009), labor timing is also surmised to be regulated by the miRNAs present in EVs derived from placenta and umbilical artery. Related to this hypothesis, three studies included in this review compared the miRNA expression profile in EVs isolated from blood plasma and Primary Human Trophoblast (PHT) cells between women with preterm and with full-term labors (Fallen *et al.* 2018; Menon *et al.* 2019; Yadava *et al.* 2021). A dysregulation in the miRNA expression profile of EVs was found in preterm birth patients compared to full-term pregnancies. Fallen et al. (2018) analyzed more than 500 miRNA and indicated that nearly 50% belonged to the placental expression of *C19MC*, which reflects the overall health status in the placenta. The genes targeted by most of the dysregulated miRNAs found in blood plasma of women who had preterm birth were described to be related to cell proliferation and focal adhesion molecules, affecting PI3K, AKT and VEGF signaling pathways (Fallen *et al.* 2018). Another study suggested that the upregulation of miR-15b-5p in EVs released from PHT cells could be an interesting biomarker for preterm birth (Yadava *et al.* 2021). Since the predicted target gene of miR-15b-5p was *APLN*, its repression is known to upregulate proinflammatory cytokines in the placenta, resulting in several processes regarding homeostasis, cardiovascular function and regulating cell apoptosis and oxidative stress regulation (Briana and Malamitsi-Puchner 2009). As previously stated, five miRNAs were commonly dysregulated both in preterm birth and in preeclampsia, thus suggesting that both affectations can be somehow related to them. These miRNAs encapsulated within EVs, therefore, could be considered as putative biomarkers of these pathologies.

Gestational diabetes mellitus

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

Strengths and limitations

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

Conclusions

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

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

Author contributions

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

REFERENCES

Abu-Halima M., Ludwig N., Hart M., Leidinger P., Backes C., Keller A., Hammadeh M., and Meese E. (2016). Altered micro-ribonucleic acid expression profiles of extracellular microvesicles in the seminal plasma of patients with oligoasthenozoospermia. *Fertil. Steril.* **106**, 1061-1069.e3. doi:10.1016/j.fertnstert.2016.06.030

Afanasyeva E. A., Mestdagh P., Kumps C., Vandesompele J., Ehemann V., Theissen J., Fischer M., Zapatka M., Brors B., Savelyeva L., Sagulenko V., Speleman F., Schwab M., and Westermann F. (2011). MicroRNA miR-885-5p targets CDK2 and MCM5, activates p53 and inhibits proliferation and survival. *Cell Death Differ.* **18**, 974–984. doi:10.1038/cdd.2010.164

Andronico F., Battaglia R., Ragusa M., Barbagallo D., Purrello M., and Di Pietro C. (2019). Extracellular vesicles in human oogenesis and implantation. *Int. J. Mol. Sci.* **20**,. doi:10.3390/ijms20092162

Aqrawi L. A., Galtung H. K., Vestad B., Øvstebø R., Thiede B., Rusthen S., Young A., Guerreiro E. M., Utheim T. P., Chen X., Utheim Ø. A., Palm Ø., and Jensen J. L. (2017). Identification of potential saliva and tear biomarkers in primary Sjögren's syndrome, utilising the extraction of extracellular vesicles and proteomics analysis. *Arthritis Res. Ther.* **19**, 1–15. doi:10.1186/s13075-017-1228-x

Barceló M., Mata A., Bassas L., and Larriba S. (2018). Exosomal microRNAs in seminal plasma are markers of the origin of azoospermia and can predict the presence of sperm in testicular tissue. *Hum. Reprod.* **33**, 1087–1098. doi:10.1093/humrep/dey072

Baskaran S., Panner Selvam M. K., and Agarwal A. (2020). 'Exosomes of male reproduction.' (Elsevier Inc.) doi:10.1016/bs.acc.2019.08.004

Battaglia R., Musumeci P., Ragusa M., Barbagallo D., Scalia M., Zimbone M., Lo Faro M. J., Borzì P., Scollo P., Purrello M., Vento M. E., and Di Pietro C. (2020). Ovarian aging increases small extracellular vesicle CD81+ release in human follicular fluid and influences miRNA profiles. *Aging (Albany. NY).* **12**, 12324–12341. doi:10.18632/aging.103441

Bechoua S., Rieu I., Sion B., and Grizard G. (2011). Prostasomes as potential modulators of tyrosine phosphorylation in human spermatozoa. *Syst. Biol. Reprod. Med.* **57**, 139–148. doi:10.3109/19396368.2010.549538

Biró O., Alasztics B., Molvarec A., Joó J., Nagy B., and Rigó J. (2017). Various levels of circulating exosomal total-miRNA and miR-210 hypoxamiR in different forms of pregnancy hypertension. *Pregnancy Hypertens.* **10**, 207–212. doi:10.1016/j.preghy.2017.09.002

Biró O., Fóthi Á., Alasztics B., Nagy B., Orbán T. I., and Rigó J. (2019). Circulating exosomal and Argonaute-bound microRNAs in preeclampsia. *Gene* **692**, 138–144. doi:10.1016/j.gene.2019.01.012

Borges E., Mulato M. G. F., Setti A. S., Iaconelli A., Geraldo M. V., and Braga D. P. de A. F. (2020). Serum microRNA profiling for the identification of predictive molecular markers of the response to controlled ovarian stimulation. *J. Bras. Reprod. Assist.* **24**, 97–103. doi:10.5935/1518-0557.20190070

Brennan K., Martin K., FitzGerald S. P., O'Sullivan J., Wu Y., Blanco A., Richardson C., and Mc Gee M. M. (2020). A comparison of methods for the isolation and separation of extracellular vesicles from protein and lipid particles in human serum. *Sci. Rep.* **10**, 1039. doi:10.1038/s41598-020-57497-7

Briana D. D., and Malamitsi-Puchner A. (2009). Reviews: Adipocytokines in normal and complicated pregnancies. *Reprod. Sci.* **16**, 921–937.

doi:10.1177/1933719109336614

Buschmann D., Kirchner B., Hermann S., Märte M., Wurmser C., Brandes F., Kotschote S., Bonin M., Steinlein O. K., Pfaffl M. W., Schelling G., and Reithmair M. (2018). Evaluation of serum extracellular vesicle isolation methods for profiling miRNAs by next-generation sequencing. *J. Extracell. Vesicles* **7**, 1481321. doi:10.1080/20013078.2018.1481321

Carson S. A., and Kallen A. N. (2021). Diagnosis and Management of Infertility: A Review. *JAMA* **326**, 65–76. doi:10.1001/jama.2021.4788

Chen Y., Wang K., Xu Y., Guo P., Hong B., Cao Y., Wei Z., Xue R., Wang C., and Jiang H. (2019). Alteration of Myeloid-Derived Suppressor Cells, Chronic Inflammatory Cytokines, and Exosomal miRNA Contribute to the Peritoneal Immune Disorder of Patients With Endometriosis. *Reprod. Sci.* **26**, 1130–1138. doi:10.1177/1933719118808923

Colombo M., Raposo G., and Théry C. (2014). Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell Dev. Biol.* **30**, 255–289. doi:10.1146/annurev-cellbio-101512-122326

Cronqvist T., Tannetta D., Mörgelin M., Belting M., Sargent I., Familari M., and Hansson S. R. (2017). Syncytiotrophoblast derived extracellular vesicles transfer functional placental miRNAs to primary human endothelial cells. *Sci. Rep.* **7**, 1–14. doi:10.1038/s41598-017-04468-0

Diez-Fraile A., Lammens T., Tilleman K., Witkowski W., Verhasselt B., De Sutter P., Benoit Y., Espeel M., and D’Herde K. (2014). Age-associated differential microRNA levels in human follicular fluid reveal pathways potentially determining fertility and success of in vitro fertilization. *Hum. Fertil.* **17**, 90–98. doi:10.3109/14647273.2014.897006

European IVF-monitoring Consortium (EIM)‡ for the European Society of Human Reproduction and Embryology (ESHRE) W., Wyns C., Bergh C., Calhaz-Jorge C., De Geyter C., Kupka M. S., Motrenko T., Rugescu I., Smeenk J., Tandler-Schneider A., Vidakovic S., and Goossens V. (2020). ART in Europe, 2016: results generated from European registries by ESHRE. *Hum. Reprod. open* **2020**, hoaa032. doi:10.1093/hropen/hoaa032

Fallen S., Baxter D., Wu X., Kim T. K., Shynlova O., Lee M. Y., Scherler K., Lye S., Hood L., and Wang K. (2018). Extracellular vesicle RNAs reflect placenta dysfunction and are a biomarker source for preterm labour. *J. Cell. Mol. Med.* **22**, 2760–2773. doi:10.1111/jcmm.13570

Feig D. S., Berger H., Donovan L., Godbout A., Kader T., Keely E., and Ma R. S. (2018). 2018 Clinical Practice Guidelines Diabetes and Pregnancy Diabetes Canada Clinical Practice Guidelines Expert Committee Pre-Existing Diabetes Preconception and During Pregnancy. *Can J Diabetes* **42**, S255-282.

Foot N. J., and Kumar S. (2021). The Role of Extracellular Vesicles in Sperm Function and Male Fertility. *Subcell. Biochem.* **97**, 483–500. doi:10.1007/978-3-030-67171-6_19

Foster B. P., Balassa T., Benen T. D., Dominovic M., Elmadjian G. K., Florova V., Fransolet M. D., Kestlerova A., Kmiecik G., Kostadinova I. A., Kyvelidou C., Meggyes M., Mincheva M. N., Moro L., Pastuschek J., Spoldi V., Wandernoth P., Weber M., Toth B., and Markert U. R. (2016). Extracellular vesicles in blood, milk and body fluids of the female and male urogenital tract and with special regard to reproduction. *Crit. Rev. Clin. Lab. Sci.* **53**, 379–395. doi:10.1080/10408363.2016.1190682

Galley J. D., and Besner G. E. (2020). The therapeutic potential of breast milk-derived extracellular vesicles. *Nutrients* **12**, 745. doi:10.3390/nu12030745

Gardner D. K., and Balaban B. (2016). Assessment of human embryo development using morphological criteria in an era of time-lapse, algorithms and ‘OMICS’: is looking good still important? *Mol. Hum. Reprod.* **22**, 704–718. doi:10.1093/molehr/gaw057

Gillet V., Ouellet A., Stepanov Y., Rodosthenous R. S., Croft E. K., Brennan K., Abdelouahab N., Baccarelli A., and Takser L. (2019). MiRNA Profiles in Extracellular Vesicles from Serum Early in Pregnancies Complicated by Gestational Diabetes Mellitus. *J. Clin. Endocrinol. Metab.* **104**, 5157–5169. doi:10.1210/jc.2018-02693

Giudice L. C. (2010). Endometriosis (DL Olive, Ed.). *N. Engl. J. Med.* **362**, 2389–2398. doi:10.1056/NEJMcp1000274

Hromadnikova I., Dvorakova L., Kotlabova K., and Krofta L. (2019). The prediction of gestational hypertension, preeclampsia and fetal growth restriction via the first trimester screening of plasma exosomal C19MC microRNAs. *Int. J. Mol. Sci.* **20**,. doi:10.3390/ijms20122972

Hu J., Tang T., Zeng Z., Wu J., Tan X., and Yan J. (2020). The expression of small RNAs in exosomes of follicular fluid altered in human polycystic ovarian syndrome. *PeerJ* **2020**,. doi:10.7717/peerj.8640

Huang J., Zheng L., Wang F., Su Y., Kong H., and Xin H. (2020). Mangiferin ameliorates placental oxidative stress and activates PI3K/Akt/mTOR pathway in mouse model of preeclampsia. *Arch. Pharm. Res.* **43**, 233–241. doi:10.1007/s12272-020-01220-7

Huppertz B., Kadyrov M., and Kingdom J. C. P. (2006). Apoptosis and its role in the trophoblast. *Am. J. Obstet. Gynecol.* **195**, 29–39. doi:10.1016/j.ajog.2005.07.039

Ji L., Xu R., Lu L., Zhang J., Yang G., Huang J., Wu C., and Zheng C. (2013). TM6, a novel nuclear matrix attachment region, enhances its flanking gene expression through influencing their chromatin structure. *Mol. Cells* **36**, 127–37. doi:10.1007/s10059-013-0092-z

Johnstone R. ., Mathew A., Mason A. ., and Teng K. (1991). Exosome Formation During Maturation of Mammalian and Avian Reticulocytes: Evidence That Exosome Release Is a Major Route for Externalization. **2736**,.

Khalaj K., Miller J. E., Lingegowda H., Fazleabas A. T., Young S. L., Lessey B. A., Koti M., and Tayade C. (2019). Extracellular vesicles from endometriosis patients are characterized by a unique miRNA-lncRNA signature. *JCI Insight* **4**,. doi:10.1172/jci.insight.128846

Krysko D. V., Diez-Fraile A., Criel G., Svistunov A. A., Vandenabeele P., and D'Herde K. (2008). Life and death of female gametes during oogenesis and folliculogenesis. *Apoptosis* **13**, 1065–1087. doi:10.1007/s10495-008-0238-1

Kuklina E. V., Ayala C., and Callaghan W. M. (2009). Hypertensive disorders and severe obstetric morbidity in the united states. *Obstet. Gynecol.* **113**, 1299–1306. doi:10.1097/AOG.0b013e3181a45b25

Leaver R. B. (2016). Male infertility: an overview of causes and treatment options. *Br. J. Nurs.* **25**, 35–41.

Lee D. C., Romero R., Kim J. S., Tarca A. L., Montenegro D., Pineles B. L., Kim E., Lee J., Kim S. Y., Draghici S., Mittal P., Kusanovic J. P., Chaiworapongsa T., Hassan S. S., and Kim C. J. (2011). MiR-210 targets iron-sulfur cluster scaffold homologue in human trophoblast cell lines: Siderosis of interstitial trophoblasts as a novel pathology of preterm preeclampsia and small-for-gestational-age pregnancies. *Am. J. Pathol.* **179**, 590–602. doi:10.1016/j.ajpath.2011.04.035

Li T., Greenblatt E. M., Shin M. E. J., Brown T. J., and Chan C. (2020). Cargo small non-coding RNAs of extracellular vesicles isolated from uterine fluid associate with endometrial receptivity and implantation success. *Fertil. Steril.* 1–9. doi:10.1016/j.fertnstert.2020.10.046

Liberati A., Altman D. G., Tetzlaff J., Mulrow C., Gøtzsche P. C., Ioannidis J. P. A., Clarke M., Devereaux P. J., Kleijnen J., and Moher D. (2009). The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. *PLoS Med.* **6**, e1000100. doi:10.1371/journal.pmed.1000100

Machtinger R., Laurent L. C., and Baccarelli A. A. (2016). Extracellular vesicles: Roles in gamete maturation, fertilization and embryo implantation. *Hum. Reprod. Update* **22**, 182–193. doi:10.1093/humupd/dmv055

Machtinger R., Rodosthenous R. S., Adir M., Mansour A., Racowsky C., Baccarelli A. A., and Hauser R. (2017). Extracellular microRNAs in follicular fluid and their potential association with oocyte fertilization and embryo quality: an exploratory study. *J. Assist. Reprod. Genet.* **34**, 525–533. doi:10.1007/s10815-017-0876-8

Martinez R. M., Baccarelli A. A., Liang L., Dioni L., Mansur A., Adir M., Bollati V., Racowsky C., Hauser R., and Machtinger R. (2019). Body mass index in relation to extracellular vesicle–linked microRNAs in human follicular fluid. *Fertil. Steril.* **112**, 387-396.e3. doi:10.1016/j.fertnstert.2019.04.001

Martinez R. M., Liang L., Racowsky C., Dioni L., Mansur A., Adir M., Bollati V., Baccarelli A. A., Hauser R., and Machtinger R. (2018). Extracellular microRNAs profile in human follicular fluid and IVF outcomes. *Sci. Rep.* **8**, 1–10. doi:10.1038/s41598-018-35379-3

Mendelson C. R. (2009). Minireview: Fetal-maternal hormonal signaling in pregnancy and labor. *Molecular Endocrinology* **23**, 947–954. doi:10.1210/me.2009-0016

Menon R., Debnath C., Lai A., Guanzon D., Bhatnagar S., Kshetrapal P. K., Sheller-

Miller S., and Salomon C. (2019). Circulating exosomal miRNA profile during term and preterm birth pregnancies: A longitudinal study. *Endocrinology* **160**, 249–275. doi:10.1210/en.2018-00836

Motawi T. M. k., Sabry D., Maurice N. W., and Rizk S. M. (2018). Role of mesenchymal stem cells exosomes derived microRNAs; miR-136, miR-494 and miR-495 in pre-eclampsia diagnosis and evaluation. *Arch. Biochem. Biophys.* **659**, 13–21. doi:10.1016/j.abb.2018.09.023

Nair S., Jayabalan N., Guanzon D., Palma C., Scholz-Romero K., Elfeky O., Zuñiga F., Ormazabal V., Diaz E., Rice G. E., Duncombe G., Jansson T., McIntyre H. D., Lappas M., and Salomon C. (2018). Human placental exosomes in gestational diabetes mellitus carry a specific set of miRNAs associated with skeletal muscle insulin sensitivity. *Clin. Sci.* **132**, 2451–2467. doi:10.1042/CS20180487

Ospina-Prieto S., Chaiwangyen W., Herrmann J., Groten T., Schleussner E., Markert U. R., and Morales-Prieto D. M. (2016). MicroRNA-141 is upregulated in preeclamptic placentae and regulates trophoblast invasion and intercellular communication. *Transl. Res.* **172**, 61–72. doi:10.1016/j.trsl.2016.02.012

Pillay P., Vatish M., Duarte R., Moodley J., and Mackraj I. (2019). Exosomal microRNA profiling in early and late onset preeclamptic pregnant women reflects pathophysiology. *Int. J. Nanomedicine* **14**, 5637–5657. doi:10.2147/IJN.S208865

Pons-Rejraji H., Artonne C., Sion B., Brugnon F., Canis M., Janny L., and Grizard G. (2011). Prostasomes: Inhibitors of capacitation and modulators of cellular signalling in human sperm. *Int. J. Androl.* **34**, 568–580. doi:10.1111/j.1365-2605.2010.01116.x

Rodosthenous R. S., Burris H. H., Sanders A. P., Just A. C., Dereix A. E., Svensson K., Solano M., Téllez-Rojo M. M., Wright R. O., and Baccarelli A. A. (2017). Second trimester extracellular microRNAs in maternal blood and fetal growth: An exploratory study. *Epigenetics* **12**, 804–810. doi:10.1080/15592294.2017.1358345

Ronquist G. (2012). Prostasomes are mediators of intercellular communication: from basic research to clinical implications. *J. Intern. Med.* **271**, 400–13. doi:10.1111/j.1365-2796.2011.02487.x

Rooda I., Hasan M. M., Roos K., Viil J., Andronowska A., Smolander O.-P., Jaakma Ü., Salumets A., Fazeli A., and Velthut-Meikas A. (2020). Cellular, Extracellular and Extracellular Vesicular miRNA Profiles of Pre-Ovulatory Follicles Indicate Signaling Disturbances in Polycystic Ovaries. *Int. J. Mol. Sci.* **21**, 9550. doi:10.3390/ijms21249550

Salas-Huetos A., James E. R., Aston K. I., Carrell D. T., Jenkins T. G., and Yeste M. (2020). The role of miRNAs in male human reproduction: a systematic review. *Andrology* **8**, 7–26. doi:10.1111/andr.12714

Salomon C., Guanzon D., Scholz-Romero K., Longo S., Correa P., Illanes S. E., and Rice G. E. (2017). Placental exosomes as early biomarker of preeclampsia: Potential role of exosomalmicrornas across gestation. *J. Clin. Endocrinol. Metab.* **102**, 3182–3194. doi:10.1210/jc.2017-00672

Sandrim V. C., Luizon M. R., Palei A. C., Tanus-Santos J. E., and Cavallie R. C. (2016). Circulating microRNA expression profiles in pre-eclampsia: Evidence of increased miR-885-5p levels. *BJOG An Int. J. Obstet. Gynaecol.* **123**, 2120–2128. doi:10.1111/1471-0528.13903

Sang Q., Yao Z., Wang H., Feng R., Wang H., Zhao X., Xing Q., Jin L., He L., Wu L., and Wang L. (2013). Identification of MicroRNAs in human follicular fluid: Characterization of MicroRNAs that govern steroidogenesis in vitro and are associated with polycystic ovary syndrome in vivo. *J. Clin. Endocrinol. Metab.* **98**, 3068–3079. doi:10.1210/jc.2013-1715

Simon C., Greening D. W., Bolumar D., Balaguer N., Salamonsen L. A., and Vilella F. (2018). Extracellular vesicles in human reproduction in health and disease. *Endocr. Rev.* **39**, 292–332. doi:10.1210/er.2017-00229

Sullivan R. (2016). Epididymosomes: Role of extracellular microvesicles in sperm maturation. *Front. Biosci. (Schol. Ed).* **8**, 106–114. doi:10.2741/S450

Sullivan R., and Saez F. (2013). Epididymosomes, prostasomes, and liposomes: their roles in mammalian male reproductive physiology. *REPRODUCTION* **146**, R21–R35. doi:10.1530/REP-13-0058

Sullivan R., Saez F., Girouard J., and Frenette G. (2005). Role of exosomes in sperm maturation during the transit along the male reproductive tract. *Blood Cells, Mol. Dis.* **35**, 1–10. doi:10.1016/j.bcmd.2005.03.005

Trams E. G., Lauter C. J., Salem N., and Heine U. (1981). Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. *Biochim. Biophys. Acta* **645**, 63–70. doi:10.1016/0005-2736(81)90512-5

Truong G., Guanzon D., Kinhal V., Elfeky O., Lai A., Longo S., Nuzhat Z., Palma C., Scholz-Romero K., Menon R., Mol B. W., Rice G. E., and Salomon C. (2017). Oxygen tension regulates the miRNA profile and bioactivity of exosomes released from extravillous trophoblast cells-Liquid biopsies for monitoring complications of pregnancy. *PLoS One* **12**, 1–27. doi:10.1371/journal.pone.0174514

Valadi H., Ekström K., Bossios A., Sjöstrand M., Lee J. J., and Lötvall J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* **9**, 654–659. doi:10.1038/ncb1596

Vojtech L., Woo S., Hughes S., Levy C., Ballweber L., Sauteraud R. P., Strobl J., Westerberg K., Gottardo R., Tewari M., and Hladik F. (2014). Exosomes in human semen carry a distinctive repertoire of small non-coding RNAs with potential regulatory functions. *Nucleic Acids Res.* **42**, 7290–7304. doi:10.1093/nar/gku347

Vyas P., Balakier H., and Librach C. L. (2019). Ultrastructural identification of CD9 positive extracellular vesicles released from human embryos and transported through the zona pellucida. *Syst. Biol. Reprod. Med.* **65**, 273–280. doi:10.1080/19396368.2019.1619858

Wang Y., Du X., and Wang J. (2020). Transfer of miR-15a-5p by placental exosomes promotes pre-eclampsia progression by regulating PI3K/AKT signaling pathway via CDK1. *Mol. Immunol.* **128**, 277–286. doi:10.1016/j.molimm.2020.10.019

Wu L., Ding Y., Han S., and Wang Y. (2020). Role of Exosomes in the Exchange of Spermatozoa after Leaving the Seminiferous Tubule: A Review. *Curr. Drug Metab.* **21**, 330–338. doi:10.2174/1389200221666200520091511

Xu L., Wu L.-F., and Deng F.-Y. (2019). Exosome: An Emerging Source of Biomarkers for Human Diseases. *Curr. Mol. Med.* **19**, 387–394. doi:10.2174/1566524019666190429144310

Xueya Z., Yamei L., Sha C., Dan C., Hong S., Xingyu Y., and Weiwei C. (2020). Exosomal encapsulation of miR-125a-5p inhibited trophoblast cell migration and proliferation by regulating the expression of VEGFA in preeclampsia. *Biochem. Biophys. Res. Commun.* **525**, 646–653. doi:10.1016/j.bbrc.2020.02.137

Yadava S. M., Feng A., Parobchak N., Wang B., and Rosen T. (2021). miR-15b-5p promotes expression of proinflammatory cytokines in human placenta by inhibiting Apelin signaling pathway. *Placenta* **104**, 8–15. doi:10.1016/j.placenta.2020.11.002

Zhang D., Lv J., Tang R., Feng Y., Zhao Y., Fei X., Chian R., and Xie Q. (2021). Association of exosomal microRNAs in human ovarian follicular fluid with oocyte quality. *Biochem. Biophys. Res. Commun.* **534**, 468–473.

doi:10.1016/j.bbrc.2020.11.058

Zhang W., Zhou X., Zhang H., Yao Q., Liu Y., and Dong Z. (2016). Extracellular vesicles in diagnosis and therapy of kidney diseases. *American Journal of Physiology - Renal Physiology* **311**, F844–F851. doi:10.1152/ajprenal.00429.2016

Zomer A., Vendrig T., Hopmans E. S., van Eijndhoven M., Middeldorp J. M., and Pegtel D. M. (2010). Exosomes. *Commun. Integr. Biol.* **3**, 447–450. doi:10.4161/cib.3.5.12339

FIGURE CAPTIONS

Fig 1. Flowchart of the literature search and selection process.

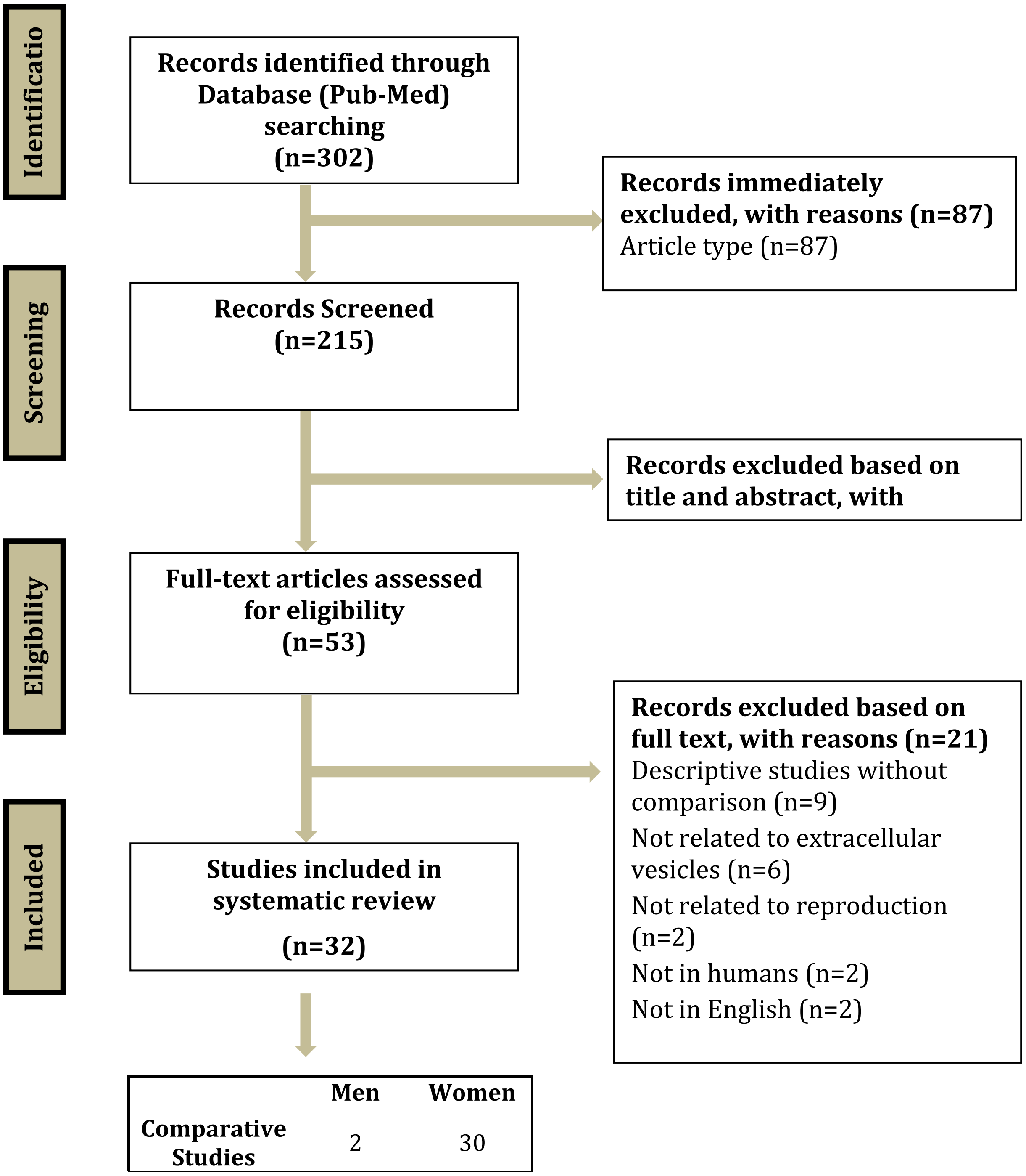

Table 1. Population, Intervention, Comparator, Outcome and Study (PICOS) design, with the inclusion and exclusion criteria and the keywords used for the definition of the search strategy and the eligibility of the study.

Parameter	***Inclusion***	***Exclusion***	***Keywords***
Population	Human (male and female)	Species other than humans	Human, *Homo sapiens*
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.

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

Table 2. *Summary of the main identified microRNAs (miRNAs) encapsulated in extracellular vesicles (EVs) extracted from the comparative studies included in the systematic 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 *et al.* 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)	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	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***	***Downregulated***	The study demonstrated an altered miRNA expression profile of EVs in seminal plasma from oligoasthenozoospermic subfertile men compared to normozoospermic fertile men.
						miR-1275 miR-4298 miR-3675-3p miR-765 miR-483-5p miR-1299 miR-766	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	
(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***	***Downregulated***	The study demonstrated a miRNA signature contained within EVs
						hsa-miR-206 hsa-miR-29c-3p	hsa-miR-1266-5p hsa-miR-200c-3p	

	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 *et al.* 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 *et al.* 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 *in vitro* 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 *et al.* 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 *et al.* 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***	***Downregulated***	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
						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	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	
(Rooda *et al.* 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***	***Downregulated***	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
						hsa-miR-200c-3p hsa-miR-100-5p hsa-miR-10a-5p hsa-miR-342-3p hsa-miR-28-3p hsa-miR-125b-5p	hsa-miR-17-5p	
(Sang *et al.* 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***	The study demonstrated that there are several miRNAs in follicular fluid some of them play a key roles in steroidogenesis and polycystic ovarian syndrome
							miR-132 miR-320	
(Martinez *et al.* 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***	***Downregulated***	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
						Fertilization status		
						hsa-miR-92a hsa-miR-130b	--	
						Poor VS high quality		
						hsa-miR-888	hsa-miR-214	

							hsa-miR-454	molecular mechanisms that could lead to a successful pregnancy and birth
(Zhang *et al.* 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***	***Downregulated***	The study indicated that the dysregulated miRNAs contained within EVs isolated from follicular fluid may be potential biomarkers for evaluating oocyte quality.
						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	hsa-miR-513c-5p hsa-miR-548au-3p	
(Machtinger *et al.* 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])		***Upregulated***	***Downregulated***	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.
					Fertilization status: failed to fertilize VS fertilized	***Not fertilized VS normally fertilized***		
						--	miR-202-5p miR-206 miR-16-1-3p miR-1244	
					Day 3 Embryo quality: poor quality embryo VS high quality embryo	***Poor VS high quality***		
							miR-663b miR-766-3p miR-132-3p hsa-miR-16-5p	
(Li *et al.* 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***	***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.
						hsa-miR-362-3p	--	
(Hromadnikova *et al.* 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^{TM} Exosome Isolation Kit-[Exiqon])	Women with pregnancies complicated by preeclampsia and/or fetal growth restriction VS women with normal pregnancies (control)	***Upregulated***	***Downregulated***	This study indicated that the miRNAs contained within EVs released to the systemic circulation by the placenta may be used as a
							miR-517-5p	

	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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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)	***Upregulated***	***Downregulated***	This study identified EVs-miRNAs signatures in early onset preeclampsia and late onset preeclampsia involved in the regulation of preeclampsia associated processes
						Early onset Preeclampsia VS Control		
						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		
						Late onset Preeclampsia VS Control		
						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 *et al.* 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)	***Upregulated***	***Downregulated***	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 *et al.* 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)	***Upregulated***	***Downregulated***	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 TNFα from endothelial cells, which seems to be related to preeclampsia and preterm birth pathophysiology
						Preeclampsia VS with normal pregnancies		
						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	
						Preterm birth compared with normal pregnancies		

						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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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 *et al.* 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)	***Upregulated*** miR-6727-5p	***Downregulated*** 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.

							miR-15b-5p miR-376c-3p	
(Gillet *et al.* 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)	***Upregulated***	***Upregulated*** 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 *et al.* 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)	***Upregulated*** 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	***Downregulated*** 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 *et al.* 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.	***Upregulated*** hsa-miR-328	***Downregulated***	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 *et al.* 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	***Upregulated***	***Downregulated***	The study suggested that EVs-miRNAs circulating in blood plasma in pregnant women at second trimester were associated with fetal growth
						Small fetal growth VS appropriate fetal growth		
						--	miR-20b-5p miR-942-5p miR-324-3p miR-223-5p miR-127-3p	
						Large fetal growth VS appropriate fetal growth		
						miR-661 miR-197-3p miR-212-3p	--	