Review Article

Embarking on life’s blueprint: investigating the crucial involvement of extracellular vesicles in embryo development

Seok Hee Lee, DEGREE

Department of Obstetrics and Gynecology, Center for Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA

Running title:

Received: 2024.03.25.

Revised: 2024.06.14.

Accepted: 2024.07.29.

Corresponding author: Seok Hee Lee, DEGREE

Department of Obstetrics and Gynecology, Center for Reproductive Sciences, University of California San Francisco, San Francisco, CA 94143, USA
E-mail: seokhee.lee@ucsf.edu

https://orcid.org/
ABSTRACT

Extracellular vesicles (EVs) contain biological molecules, such as proteins, lipids, and diverse nucleic acids, which alter various physiological and pathological processes in recipient cells. This review examines the current understanding of EVs in terms of their biological characteristics, effects on embryonic development, and potential therapeutic value in treating reproductive disorders. EVs play a crucial role in early embryonic development, from fertilization to the pre-implantation stage, as well as during gastrulation, cell differentiation, and organogenesis. In the pre-implantation period, EVs interact with maternal reproductive tissue and promote implantation receptivity, whereas during gastrulation, they regulate cell differentiation and contribute to tissue formation and maintenance. Abnormal bioactive molecules in EVs are associated with developmental disorders; therefore, EVs can serve as biomarkers. In addition, EVs are potential therapeutic agents that can deliver genetic material to targeted tissues or organs. The findings of this review highlight the potential role of EVs in intercellular signaling during embryonic development. This will help advance assisted reproductive technologies and therapies to overcome infertility and developmental disorders.

Keywords: Extracellular vesicle; Exosomes, Embryo development; Reproductive disorders
Introduction

Extracellular vesicles (EVs) are a heterogeneous group of small, spherical, and lipid bilayer-enclosed structures that are released into the extracellular environment by most cell types [1-3]. They are composed of exosomes, microvesicles, and apoptotic bodies and serve as prime mediators of intercellular communication to promote the transfer of bioactive cargo between cells [4,5]. EVs vary in size, originate from internal compartments within cells, and contain diverse biomolecules such as proteins, lipids, and nucleic acids to elicit distinct biological effects on recipient cells [4,6]. These vesicles play a crucial role in regulating many physiological and pathological processes in recipient cells, including coagulation, inflammation, immune responses, tissue repair, and reproduction [2,7-10]. Among EVs, exosomes are potent paracrine mediators that facilitate cellular crosstalk and are found in various biological fluids.

By understanding the physiological characteristics of EVs, including their composition, biogenesis, and function, valuable insights into intercellular communication can be obtained. This knowledge can then be applied to develop diagnostic and therapeutic applications across various medical fields.

The formation of multicellular organisms through embryonic development is a complex process that involves biological processes such as gamete fertilization and tissue and organ development [2]. Once the developing embryo migrates to the uterus, the blastocyst must appose and subsequently adhere to the endometrial epithelium to ensure successful implantation, after which it invasively
interacts with the endometrial tissue [11]. The role of EVs in embryonic development has been extensively investigated in various scientific fields, and their identification in reproductive biofluids suggests that they are required for intercellular communication to establish successful embryo development during and after conception [12,13].

Alterations in EV signaling may be crucial factors in certain pathological conditions; therefore, understanding the molecular mechanisms of normal embryonic development induced by EVs could be beneficial for diagnosing and treating developmental disorders [14,15]. The present mini-review focuses on enhancing the current understanding of the role of EVs in early embryonic development and implantation (Fig. 1). In addition, current knowledge regarding the implications of EVs on developmental disorders and their therapeutic effects on reproductive disorders was explored.

**Cellular communication through extracellular vesicles**

Intercellular signaling mechanisms are pivotal for coordinating cellular activities and ensuring the proper progression of various biological processes, including embryonic development [16]. EVs have emerged as essential mediators of intercellular communication within this complex network, particularly in terms of their recognition and binding to the surface receptors of recipient cells [15,17]. Subsequently, internalization processes, such as endocytosis, transfer vesicular cargo into recipient cells, and thereby initiating a cascade of molecular events [18].

EVs play a role in molecular communication between cells. MicroRNAs (miRNAs) modulate gene
expression in recipient cells by influencing messenger RNA (mRNA) stability or translation [19-21], while proteins and lipids found in the vesicular cargo have distinct functions, such as activating signaling cascades and maintaining the structure of the vesicle membrane [22,23]. The packaging of these molecules into EVs allows for the precise transport of bioactive materials and signals that are required for cellular responses and normal developmental processes. Therefore, knowledge of the interactions between intercellular signaling mechanisms and molecular cargo transfer that are mediated by EVs is crucial for understanding the regulatory networks that govern embryonic development.

**Extracellular vesicles in early embryo formation**

EVs play an essential role in specific stages of embryonic development, including pre-implantation and gastrulation [1,2,24-26]. In the pre-implantation stage, EVs are involved in critical cellular interactions during the initial stages of embryogenesis before implantation into the uterine lining [27-29]. For example, exosomes derived from embryonic and maternal cells communicate with each other to establish a receptive environment for implantation [30].

Recent studies in mice have demonstrated the ability of embryo-derived exosomes to freely traverse the zona pellucida, indicating bidirectional flux [31]. Moreover, larger EVs may be engaged in penetrating the zona pellucida during blastocyst hatching. This may represent a feasible strategy for producing larger quantities of accessible materials and facilitating non-invasive assessments [27].

EVs, particularly exosomes, are rich in proteins that are crucial for cell signaling. Notable
components include the major histocompatibility complex class II, tetraspanins (CD37, CD53, CD63, CD81, and CD82), endosomal sorting complex proteins (Alix and TSG101), and various glycoproteins [32-34]. Bovine oviductal EVs express markers similar to those of general EVs, with the unique presence of the oviduct-specific protein, OVGP1. These EVs carry proteins that are involved in diverse functions, such as cell metabolism and immunomodulation [35], and co-incubation of oviductal EVs with embryos enhances blastocyst yield and quality, leading to extended embryo survival \textit{in vitro}.

A recent study demonstrated that exosomes derived from human trophoblasts contain high concentrations of placenta-specific miRNAs, which facilitate cell-to-cell communication in an \textit{in vitro} model system [36]. Furthermore, RNA sequencing analysis identified 118 differentially expressed miRNAs in exosomes obtained from healthy cows or those with endometritis [37]. This suggests that exosomal miRNAs can cause infertility in cows by influencing embryonic development and fertility during periods of uterine inflammation. Therefore, the interaction between embryonic EVs and the maternal environment during pre-implantation affects subsequent development. Table 1 lists recent studies on the effect of EVs on embryo development during pre-implantation.

In the subsequent phase of gastrulation, EVs continue to play a crucial role in cell signaling processes that support cell migration, differentiation, and distinct tissue layer formation, including the generation of embryonic germ layers [38,39]. Exosomes are key regulators of these events and they have been shown to transport morphogens and signaling molecules that determine cell fate and spatial organization during gastrulation in \textit{Xenopus} embryos [24]. Exosome complex components 1 and 2 are
proteins within the RNA exosome complex that are primarily responsible for 5′ to 3′ RNA degradation and processing. Exosome complex component 1 in homozygous null mouse embryos showed delayed development and failed gastrulation initiation, whereas exosome complex component 2 null mouse embryos were lethal during the peri-implantation stages [40]. These findings highlight the importance of the exosome complex components in early mammalian development.

**Extracellular vesicles in cell differentiation and implantation**

EVs are necessary for appropriate cell differentiation during early embryonic development and play an important role in stem cell development by transporting bioactive mediators that regulate cell fate, help maintain pluripotency, and trigger differentiation processes in embryonic stem cells [41,42]. In particular, exosomes have been shown to modulate gene expression and influence the balance between self-renewal and differentiation of embryonic stem cells. The molecular cargo carried by exosomes activates signaling pathways that determine cell fate, thereby directing the development of specific cell lineages before implantation [43-46].

The complex processes of implantation and early embryo development rely greatly on communication between various cells and organs. During embryo implantation, a dynamic change occurs in the endometrium of the uterine cavity, both before and after blastocyst arrival. The receptive endometrium has historically been thought of as a passive tissue primed for embryo implantation; however, it is now understood that this tissue engages in extensive crosstalk between the embryo and
maternal compartments [47]. Several studies support the idea that implantation and early embryo development involve intricate processes that are highly dependent on cellular and organ communication [11,48]. Upon migration into the uterus, the blastocyst apposes and subsequently adheres to the luminal epithelium of the endometrium, where it engages in invasive processes that are crucial for successful implantation. EVs play a significant role in these processes. For example, a recent study demonstrated that exosomes derived from human endometrial epithelial cells (30 µg/mL) notably enhanced cell adhesion and outgrowth of human trophectoderm stem cell-derived spheroids [49]. These exosomes also increased the total cell number in mouse embryos and improved embryo implantation rates compared to other secretome fractions, thereby underscoring their critical role in promoting successful implantation.

Successful implantation requires a coordinated interaction between the embryo and endometrium, and EVs can facilitate this crosstalk [50,51].

EVs have been shown to carry mRNAs, including OCT4, SOX2, and KLF4, with variations corresponding to different stages of embryonic development [2]. For example, exosomes containing IFNT from ovine and bovine conceptuses influence the mRNA expression of interferon-stimulated genes in uterine endometrial epithelial cells, highlighting their role in conceptus attachment and development [52,53]. Another study revealed that PMCA4 mRNA, which encodes the 4a and 4b variants, is expressed in the vagina, uterus, and oviducts of mice [51]. PMCA4a, which is found in vesicles termed "oviductosomes", is transferred to sperm through exosomal uptake in the female reproductive tract and
is crucial for regulating hyperactivated sperm motility and fertility in mice [51]. These findings establish the diverse molecular cargoes of EVs and their crucial impact on embryo-maternal communication for successful implantation.

EVs released by the endometrial epithelium may also play a role in the transport of signaling miRNAs and adhesion molecules by targeting the blastocyst or neighboring endometrium within the uterine cavity, subsequently influencing endometrial receptivity and implantation [2]. The implantation potential of embryos correlates with the levels of human embryo-derived miRNAs [54]. Specific miRNAs showed elevated levels in the media of blastocysts that were successfully implanted compared with those that were not implanted. Some of these miRNAs associated with implantation have been predicted to target pathways involved in endometrial cell growth and proliferation in humans [55]. Furthermore, a previous study examining mouse vesicles revealed that the conceptus trophectoderm and endometrial epithelium share miRNAs and proteins, including cathepsin L1 and prostaglandin synthase 2 [50]. These studies suggest that embryos release EVs and/or miRNAs that target predicted genes, thereby influencing the cellular activities essential for implantation, such as adhesion and migration.

**Implications for developmental disorders**

Maternal health is widely recognized to be closely associated with fetal development [56-58]. Recently, several studies have reported that abnormal EV content disrupts molecular signals necessary for proper
embryonic development, resulting in various developmental disorders. For example, imbalances in factors such as specific EV miRNAs have been implicated in neural tube defects and congenital heart diseases in humans [59,60]. Aberrant changes in the placental microenvironment may affect the release and content of placenta-derived EVs. A recent study demonstrated that EVs obtained from injured placentas induced preeclampsia-like symptoms in mice by inducing endothelial injury and vasoconstriction. The clearance of EVs reverses this developmental disorder, indicating the therapeutic potential of placental EV production [61]. In addition, EVs can be non-invasively isolated from maternal plasma [62] and urine [63] in humans [62] and can potentially be used as markers for preterm births. Therefore, investigating the relationship between EVs and developmental disorders could aid in early diagnosis and shed light on the molecular mechanisms that contribute to pathological outcomes during embryonic development.

EVs have recently emerged as promising therapeutic agents for the treatment of developmental disorders. Fig. 2 outlines the therapeutic effects of EVs on reproductive disorders. The delivery of genetic material or signaling molecules from EVs may offer targeted therapeutic approaches [64], which could enhance treatment in this area and incite further breakthroughs in EV-based treatments through EV engineering strategies.

**Future directions and conclusion**

Recent studies have attempted to understand the role of EVs in the reproductive microenvironment
and their impact on early embryonic events \([65,66]\). The profiles and functions of EVs have been extensively investigated in various mammals. For example, miRNAs and other EV components secreted by mammals can enhance oocyte maturation and pre-implantation embryo development \([67-74]\). However, owing to the complexity and dynamism of the human reproductive system, our understanding of the specific cargo components of EVs is relatively limited, particularly in terms of their proteomic and metabolomic profiles. A comprehensive understanding of these profiles could help identify the molecular mechanisms involved in the intricate metabolic dynamics of early embryonic development.

From a technological perspective, advancements in methodologies and tools for studying EVs are necessary to assess the precise biological responses of target tissues after EV implementation. For example, single-vesicle analysis and high-resolution imaging techniques can enable researchers to analyze the heterogeneity of EV populations, whereas advanced isolation and purification methods can improve the efficiency of EV research \([75,76]\).

In conclusion, EVs are emerging as key players in reproductive and developmental processes from the embryo to organs, and it is critical to understand their specific roles in intercellular signaling and genetic and epigenetic regulation. By expanding our knowledge of EVs in the reproductive field, we can develop new assisted reproductive technologies to overcome infertility issues, create targeted therapies for developmental disorders, and address the complex challenges in embryo development.
Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical approval

N/A.

Patient consent

N/A.

Funding information

This study was supported by the Basic Science Research Program of the National Research Foundation of Korea (2021R1A6A3A14046145).


5. Altan-Bonnet N. Extracellular vesicles are the Trojan horses of viral infection. Curr Opin Microbiol 2016;32:77-81.


18. Wan C, Stowell MHB, Shen J. Progress and gaps of extracellular vesicle-mediated intercellular cargo


40. Srinivasan S, He X, Mirza S, Mager J. Exosome complex components 1 and 2 are vital for early
mammalian development. Gene Expr Patterns 2024;51:119346.


49. Gurung S, Greening DW, Catt S, Salamonsen L, Evans J. Exosomes and soluble secretome from


**Fig. 1.** Schematic illustration of the role of extracellular vesicles in signaling pathways related to oocyte maturation and embryo development. EVs, extracellular vesicles; mRNA, messenger RNA.
**Fig. 2.** Schematic illustration of the therapeutic effects of extracellular vesicles on reproductive disorders.

EVs, extracellular vesicles; ATP, FULL NAME.
Table 1. Recent studies on the effects of extracellular vesicles (EVs) on embryo development
<table>
<thead>
<tr>
<th>Topic</th>
<th>Species</th>
<th>Finding</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Role of miRNA in oviductal fluid on embryo development</td>
<td>Bovine</td>
<td>Treatment with miR-17-5p improves embryonic development to the blastocyst stage</td>
<td>Aoki et al. [13] (2022)</td>
</tr>
</tbody>
</table>
| Effect of EVs from human fallopian tubal fluid on embryo development  | Human & murine| - Increase blastocyst and hatching rate  
- Decrease reactive oxygen species level and apoptotic cell proportions | Li et al. [12] (2023)         |
| Impact of ssc-miR-143-3p within EVs on porcine trophoblast cells     | Porcine       | - Promoted the proliferation and migration of cells by targeting glycerol-3 phosphate dehydrogenase 2 | Ding et al. [67] (2022)      |
| Impact of EVs isolated from oviductal and uterine fluid on the development and quality of bovine embryos | Bovine        | - Enhanced survival rates of blastocysts after vitrification/warming  
- Increased total cell numbers, reduced lipid content, and altered expression of lipid metabolism-related transcripts | Leal et al. [68] (2022)      |
| Role of EVs isolated from porcine oviduct fluid in improving the     | Porcine       | - Improved blastocyst formation rates and total cell numbers  
- Alleviation of endoplasmic | Fu et al. [69] (2022)          |
<table>
<thead>
<tr>
<th>embryonic development</th>
<th>reticulum stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size, concentration, and miRNA content of EVs secreted by bovine embryos during the compaction period</td>
<td>Bovine</td>
</tr>
</tbody>
</table>

Melo-Baez et al. [71] (2020)

miRNA, microRNAs; miR-17, microRNA 17; ssc-miR, FULL NAME.