

Review

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Review

From Germ Cells to Implantation: The Role of Extracellular Vesicles

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Abstract: Extracellular vesicles represent a large heterogeneous class of near and long-distance intercellular communication mediators, released by both prokaryotic and eukaryotic cells. Specifically, the scientific community has shown a growing interest in exosomes, which are nano-sized vesicles with an endosomal origin. Not so long ago, the physiological goal of exosome generation was largely unknown and required more investigation; at first, it was hypothesized that exosomes are able to remove excess, rejects and unnecessary constituents from cells to preserve cellular homeostasis. However, thanks to recent studies, the central role of exosomes in regulating cellular dialogue has emerged. Exosomes act as vectors on cell-cell signaling by their cargo, proteins, lipids, and nucleic acids, and influence physiological and pathological processes. The findings on exosomes are widespread in a large spectrum of biomedical applications from diagnosis and prognosis to therapies. In this review, we describe exosome biogenesis and the current methods for their isolation and characterization, emphasizing the role of their cargo in female reproductive processes, from gametogenesis to implantation, and the potential involvement in human female disorders.

Keywords: extracellular vesicles; exosomes; implantation; embryo; gametogenesis; germ cells; microRNAs; biomarkers; blastocoel fluid

1. Introduction

Intercellular communication is mediated by an extensive variety of signaling processes and it is indispensable for the maintenance of homeostasis and the safety of the whole organism [1,2]. Traditionally, cell-to-cell communication involved direct interactions (gap junctions), autocrine, paracrine and endocrine mechanisms as well signaling molecules targeting cellular receptors. Over the last few years, new mediators of intercellular communication in both Prokaryotic and Eukaryotic domains [3] have emerged: the extracellular vesicles (EVs). EVs have become a central hub in cellular biology acting as mediators among cells not only in physiological but also in pathological conditions [4]. EVs include a heterogeneous class of vesicles delimited by a phospholipid bilayer [5]. They are constitutively released by numerous cell types in response to specific stimuli or cellular stress factors [4,6] and transfer their contents to the target cells. Currently, several subgroups of EVs have been defined, such as small EVs, large EVs, ectosomes, extracellular particles, and exosome-like vesicles [3]. However, due to the difficulties in isolation methods and the different classification criteria, three major groups of vesicles are essentially considered. Based on their size, density, morphology, biogenesis and surface markers, they can be classified in microvesicles (MVs), apoptotic bodies and exosomes. MVs, also known as microparticles, are released by budding or shedding of the plasma membrane [7] and show a diameter > 100 nm. The apoptotic bodies, with the broadest range of diameters (50–5000 nm), are produced by cells undergoing apoptosis [8]. Exosomes, nano-sized vesicles (from 30 to 100 nm in diameter) show a characteristic cup-shaped morphology bounded by a bilayer membrane and are secreted by the most of cells both in physiological and pathological conditions [9]. The biogenesis of exosomes mainly goes across three different stages. First the

cytoplasmic membrane invaginates to form endosomes, which in turn fuse to form early endosomes (ESEs). Then, ESEs invaginate again to encapsulate the intracellular material, forming multiple small intraluminal vesicles that are further transformed into late endosomes (LSE) which later become multivesicular bodies (MVBs). Finally, MVBs fuse with the cytoplasmic membrane, releasing intraluminal vesicles (ILVs) into the extracellular environment [10,11]. Nowadays, accumulating evidence suggest that these secretory vesicles are released by all types of eukaryotic cells and are widely available in almost all bodily fluids, including urine, blood, cerebrospinal fluid, saliva, breast milk, seminal fluid and follicular fluid [12,13]. Precisely, they can function as intercellular transmitters to deliver their cargo molecules necessary for cell-to-cell communication via interaction on the cells surface, taking part in many biological processes [2]. Their intercellular trafficking includes proteins (such as annexins, tetraspanin, integrin family, proteoglycans, lectins, proteins involved in apoptosis) [14], lipids (such as cholesterol, diglycerides, sphingolipids, phospholipids, prostaglandins, leukotrienes) [15] and nucleic acids (DNA, mRNAs and non-codingRNAs) [16]. The term exosome was coined for the first time by Johnstone et al. in 1987 [17], representing the smallest unit of EVs which are delivered to the extracellular space [18]. Exosomes are usually hemispherical, circular, and elliptical cups with a concave side [19]. Despite notable progress, the characterization and quantification of exosomes remain considerable due to the complexity and lack of standard isolation methods [20,21]. Today, a set of standardized methods for exosome isolation and characterization have been widely accepted. In order to ensure reproducibility, comparability across the different studies and define stringent classification parameters, the International Society for Extracellular Vesicles (ISEV) proposed the Minimal Information for Studies of Extracellular Vesicles (MISEV) guidelines for the field in 2018. The MISEV 2018 guidelines include tables and outlines of suggested protocols and steps to follow to document specific EV-associated functional activities [22,23]. The most commonly used methods in exosome isolation are ultrafast-centrifugation, density gradients, filtration (by using filters with pore diameters of 0.8, 0.45, 0.22, and 0.1 μm) [24] and exosome extraction kits based on polyethylene glycol (PEG) precipitation [24]. While scanning and transmission electron microscopy (SEM and TEM) have been used to observe cup shape, confocal microscopy is employed for live imaging of exosomes uptake and their intracellular mobility [25]. Nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS) are also used to measure particle distribution, concentration and sizes in the range of 30–150 nm [26]. Western blot is commonly applied to detect the presence of molecular markers inside and outside exosomes. Nowadays, the international standard is the detection of three positive proteins (such as transmembrane Tetraspanins CD63, CD9 and/ or CD81, soluble protein TSG101 and cytoplasmic protein Alix) and one negative protein (endoplasmic reticulum protein Calnexin, nuclear proteins histone and Golgi protein GM130) [23,27,28]. In 2019, Zhang and Lyden optimized asymmetric-flowfield-flow fractionation (AF4) technology for separating and characterizing extracellular nanoparticles, particularly exosomes [29]. However, one of the main limitations of AF4 is that it fractionates samples based on their size, thus particles with the same hydrodynamic size but with different morphologies, surface molecules and other biophysical properties can not be separated from each other via AF4 alone [29]. In 2021, a paper published in International Journal of Nanomedicine [21], proposed the silicon nanowires (Si NW) label-free luminescent sensing system. It is functionalized with a specific antibody able to selectively bind the vesicles with CD81⁺ antigen. Tested with follicular fluid and blastocoel samples, Si NW label-free luminescent sensors showed high selectivity and sensitivity, outperforming standard mass approaches like ELISA. The functionalization processes used for these sensors make them suitable for commercial applications [21]. Initially, exosomes were underestimated as vesicles for cellular waste disposal; however, they are now recognized as crucial contributors in intercellular communication [30,31]. Exosomes are able to release their contents into the cytosol of acceptor cells by using their surface molecules' receptor-ligand interaction, endocytosis, or direct fusion with the targeting cell membrane [32]. Their bioactive cargoes, uptaken by recipient cells through different mechanisms, can influence molecular pathways and cellular function [33,34,35]. In, 2007 Valadi et al., demonstrated for the first time that, in addition of DNA, mRNAs, proteins and lipids, exosomes can transport non-coding RNAs (ncRNAs): in

particular, the authors revealed the presence of microRNAs (miRNAs) in exosomes cargo, which could be delivered to other cells, influencing their functions and gene expression. This represents a novel mechanism of genetic exchange between cells, that they proposed to call “exosomal shuttle RNA” (esRNA) [36]. Research is ongoing to better understand the sorting mechanisms and practical impacts of ncRNAs in exosomes: technological advancements in high-throughput sequencing and bioinformatic analyses are aiding in the detailed characterization of exosomal ncRNAs, emphasizing the complexity of intercellular communication and their potential for innovative diagnostic and therapeutic strategies [37]. These discoveries suggest that the investigation on circulating exosomes and their cargo could provide new opportunities for liquid biopsy in precision medicine, highlighting the potential role of exosomes as biomarkers for cancer diagnosis and prognosis prediction [38,39,37]. Exosomes showed greater advantages in liquid biopsy: in most cases, their abundance in biofluids could contribute to easier isolation of vesicles [35]; exosomes are secreted by living cells and contain several biological information from their origin cells [33,35]; they are innately stable thanks to their lipid bilayers, and thus stably circulate in both physiological conditions and in the harsh tumoral microenvironment [38]. However, one of the most significant limits for the application of exosomes in liquid biopsy is isolation with high efficiency and purity, due to their nanoscale size and intrinsic heterogeneity [40,41,42]. Ultrasensitive and precise detection is necessary for the development of exosome-based cancer diagnostics, since they represent only a small part of all exosomes in bodily fluids [38]. The great predictive potential of exosomal cargo finds application not only in cancer but also in reproductive medicine, pointing out the possibility of using the molecules transported inside exosomes as possible markers of gametes, embryo and implantation quality [43].

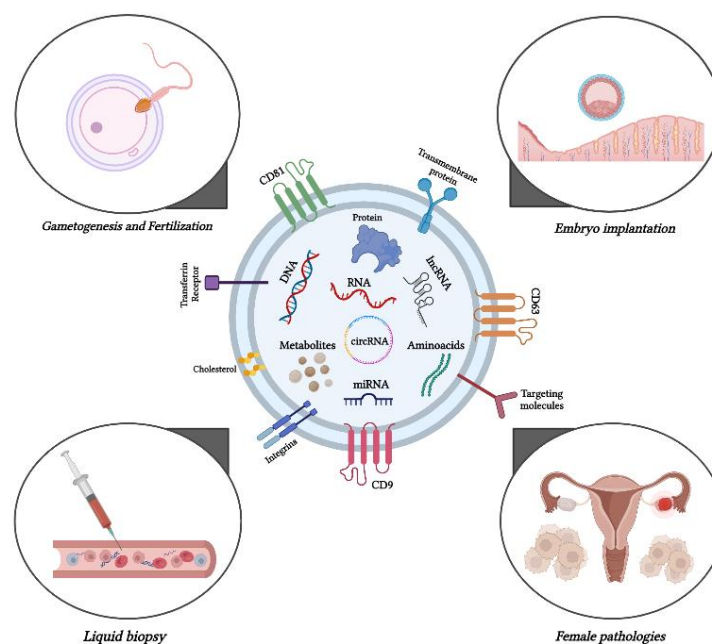


Figure 1. Graphical abstract edited by Biorender <https://www.biorender.com/> (accessed on 30 June 2024).

2. Exploring the activity of exosomes in gametogenesis and fertilization

In sexual reproduction, sperms and oocytes represent the fundamental protagonists generating a new organism. The maturation and the functionalities of germinal cells are critical points for reproductive success [44]. In the last years, several scientific evidence suggest that exosomes play significant roles in gametogenesis, facilitating communication between germ cells and the somatic cells and influencing proliferation, differentiation, and maturation of the gametes [44–48]. In spermatogenesis, the sperm maturation process and the acquisition of fertilization capability involve a series of intricate interactions along the male reproductive tract [49,50]. The Sertoli cells support and coordinate germ cell development and spermatogenesis by offering nutrients and participating

in the formation of the blood-testis barrier [51]. A few studies, reveal that Sertoli cells are able to release exosomes in the testis and allow sperm differentiation into the spermatogenic tubules [44], [52,53]. The epididymis, comprising the caput, corpus, and cauda, facilitates sperm maturation, transport, and storage: each segment offers a unique habitat optimized for different stages of sperm development [54,55]. Sperms undergo crucial changes in morphology and function during their transit through the epididymis, where they interact with different microenvironments. Throughout this transit, sperm modifies membrane composition and surface proteins, influenced by interactions with the surrounding intraluminal fluid. Numerous studies have demonstrated EVs secretion by epididymal epithelial cells (epididymosomes) and by prostate (prostasomes) [56–60]. These vesicles are able to orchestrate sperm pathways – such as capacitation, acrosome reaction, protection against oxidative stress, stability and fluidity – by transporting bioactive molecules such as sperm adhesion molecule 1 (SPAM1), glioma pathogenesis-related 1-like protein 1 (GliPr1L1), metalloprotease, proto-oncogene tyrosine-protein kinase Src (cSrc), macrophage migration inhibitory factor (MIF), dicarbonyl/L-xylulose reductase (DCXR), Liprin α 3, and also miRNAs [45,61–63]. In fact, it seems that exosome-derived miRNAs are a new component of cell-cell communication: a recent study reveal that Sertoli cell exosome-derived miR-486-5p is able to down-regulate PTEN and up-regulate the expression of Stra8, promoting the differentiation of spermatogonial stem cells (SSCs) into spermatogonia in mice [19,64]. A study conducted in 2022., [65] investigated and analyzed the protein and miRNA components of human testicular endothelial cell-derived exosomes (HTEC-Exos) revealing that the deregulation of the expression of miRNAs (hsa-miR-511, hsa-miR-222-3p, hsa-miR-320a) in sperm cells, epididymis, seminal plasma, and EVs (i.e., exosomes and microvesicles) may lead to the alterations of spermatogenesis and various forms of infertility [66]. In mammalian female gametogenesis, mature ovarian follicle, comprising oocyte, somatic cells (cumulus granulosa, mural granulosa and theca cells), and follicular fluid (FF), represent a critical dynamic microenvironment rich in metabolites and hormones to ensure follicular development and oocyte maturation [67]. Bidirectional communication between gametes and granulosa cells in the follicle occurs either directly by a network of gap junctions or through paracrine, autocrine and endocrine signalling factors in the FF [68,69]. These interactions guide to the modulation of oocyte transcriptional activity, in order to produce a competent oocyte able to support fertilization and preimplantation embryo development [70–73]. Emerging evidence highlight that exosomes contribute to this intricate interplay by transporting miRNAs that regulate several pathways involved in folliculogenesis [74], including WNT, TGF β , MAPK, ErbB, and ubiquitin-mediated signalling pathways [45,75,76]. Many studies about the involvement of EVs from FF in follicular maturation use animal models. Hung et al.[77] have demonstrated that bovine follicular EVs are up-taken by bovine or mouse cumulus cell, consequently inducing the expansion of cumulus-oocyte complex (COC) thanks to the modulation of Prostaglandin-endoperoxide synthase 2 (Ptgs2) and Pentraxin 3 (Ptx3) expression, genes known to be involved in this process [77]. EVs originating in early, mid-estrus and pre-ovulation stages of oocyte maturation in mares, present dissimilar concentrations and different miRNA contents depending on the stage. In particular, miR-125 and miR-199, probably secreted by granulosa cells and packaged in EVs, were particularly expressed in the pre-ovulatory stages, being involved in oocyte maturation and cumulus proliferation. MiR-21, miR-132, and miR-212, whose expression seems to be hCG/LH-related [10] regulate gene expression inducing granulosa cell proliferation and the maturation of the cumulus-oocyte complex [78]. In 2014, Diez-Fraile et al. [79] identified four different expressed miRNAs in EVs from FF comparing younger with older women: specifically, they found miR-99b, miR-134 and miR-190b upregulated and miR-21-5p downregulated in older women [10]. These miRNAs regulate genes involved in heparan-sulphate proteoglycan expression, carbohydrate digestion and absorption, and apoptosis and their altered regulation could affect follicle development and oocyte maturation [10,45,79]. Finally, it seems that exosomes may take part also in the fertilization process, which represents a complex regulatory mechanism involving sequential events essential for the successful merge of sperm and oocyte [45]. When sperm passes through the uterus, the uterine cell-derived exosomes (uterosomes) carry transmembrane proteins and glycosyl phosphatidylinositol junction protein (SPAM1), which are essential for sperm fertilization and

enhance the ability to cross the cumulus cell [80]. Similarly, when sperm passes through the oviduct, oocyte-derived exosomes (Oc-Exo) deliver specific sugar protein membrane Ca^{2+} -ATPase4 (PMCA4) to the sperm surface improving the resistance to zona pellucida hydrolysis and reducing multiple sperm fertilization. Besides, Oc-Exo promote spermatozoa motility and their capacitation, thanks to the induction of tyrosine phosphorylation and consequently activating the acrosome reaction [81]: in particular, Oc-Exo carry two tetraspanins - CD81 and CD9 - [44] involved in sperm-egg fusion, acting independently of each other [44,82]. Other molecules participate in the process of sperm-egg fusion, such as glutathione peroxidase-5 (GPX5), SPAM1, prostate-specific antigen (PSA), kinesin family member 5B (KIF5B), annexin A2 (ANXA2) and kallikrein 2 (KLK2) which are delivered by human semen-derived exosomes [83]. Thanks to this finely regulated communication among gametes, orchestrated by a variety of factors and signalling molecules, the development and the implantation of embryo will be able to proceed.

3. Exosomes and embryo implantation

Embryo implantation is a critical step and requires a close dialogue between the embryo and maternal tissues coordinated by molecular and physiological signalling. The embryo adhesion to receptive endometrium needs the protection of embryo from the maternal immune system before implantation, but also the maternal endometrial inflammatory response after implantation promoting embryo invasion [84–86]. During the embryo attachment, embryonic EVs seem to transport molecules potentially able to modulate the host endometrial immune system [87,88]. An increasing repertoire of uterine CD56, CD16, natural killer (uNK) cells, T lymphocytes, B cells, and dendritic cells are recruited at implantation site during embryo implant due to their ability to secrete immunosuppressive IL-10 [89,90] to protect the embryo from maternal immune attacks. Moreover, this modulation seems to depend on miRNA and protein EV cargo [91] able to influence the establishment of pregnancy, fetal development and survival during pregnancy [92,93]. Moreover, there is increasing evidence that Uterine Fluid EVs (UF-EVs) have potent effects on the maternal immune system during implantation. Nakamura et al. in two papers published in 2019 and in 2021 [94,95] reported that in cattle, during receptivity, UF-derived EVs carry miRNAs, bta-miR-98 [94] and bta-miR-26b [95], which target and negatively regulate immunoregulatory genes in endometrial recipient cells (including CTSC, IL6, CASP4, and IKBKE, and PSMC6, CD40, and IER3, respectively) [96]. Gene set enrichment analyses of downstream target genes highlighted the involvement of neutrophil activation in immune response and neutrophil-mediated immunity as potential immunomodulatory functions. Embryos are able to release some immunosuppressive molecules (as hCG, HLAG) inside EVs, inducing the production of immunosuppressive factors (e.g., IL-10 [97]) to protect themselves from maternal immune responses during the implantation process. For instance, earlier evidence suggests that mouse embryonic EVs containing progesterone-induced-blocking factor 1 (PIBF), could interact with CD4⁺ and CD8⁺ peripheral T-cells, causing IL-10 production and immunosuppression [97]. Similarly, in a study published in 2019 [98] the authors suggested that HSPE1-associated trophoblast cell line BeWo can release EVs able to suppress regulatory T-cell (Treg) signalling by modulation of miRNAs (like hsa-miR-23b, hsa-miR-146a, hsa-miR-155, hsa-miR-22, and hsa-miR-221) and protein (HSPE1) expression on the T-cell surface and lumen highlighting their potential to regulate immune cell function during embryo implantation [96]. Another study conducted by Rai et al. in 2021 [99] highlights that EVs present in human UF contain antioxidative regulators (SOD1, GSTO1, MPO, CAT) specifically during the secretory phase [99]: they are able to reduce ROS levels facilitating the embryo apposition and his attachment to the endometrium [96]. The control and regulation of the communication between the mother and developing embryo require also the contribution of the placenta. It is an essential organ performing vital functions (ensuring gas exchange, nutrient and waste transfer, immunoglobulin transport, and hormone secretion [18] for the fetus to support its growth and survival and maintain the pregnancy. The crosstalk between fetus and mother can involve the simple diffusion of molecules through tissue layers but also the extracellular vesicles, especially exosomes [93]. During pregnancy, various types of placental cells are able to secrete exosomes - placenta-derived exosomes - which concentration continuously increases

in maternal circulation over the first trimester of pregnancy [44]. They may be differentiated from other exosomes by the presence of placenta-specific growth factors, DNA fragments, mRNAs and miRNAs, which are involved in regulating the physiological function of the maternal uterus and fetal development [44]. Among the most interesting miRNAs, we can find several miRNAs located in chromosome 19 - miRNA cluster C19MC - which is the largest cluster of miRNAs in the human genome [100]. MiRNAs within the human C19MC include 46 miRNAs which are expressed during pregnancy only in the placenta, the so-called placenta-associated miRNAs [101,102], secreted into the maternal circulation by exosomes where they function in placental-maternal signalling [103,104]. For example, exosomal miR-517b increased the expression of TNF α and/or other death ligands [93], instead miR-516b-5p, miR-517-5p, and miR-518a-3p are shown to influence the PI3K-Akt and therefore the insulin signalling pathways, and their expression levels seems to be regulated by various stimuli, including oxidative stress and glucose levels [18,105]. Furthermore, the exosomal transfer of placenta-specific miR-571a-3p into NK cells repressed cGMP-dependent protein kinase 1, a crucial mediator of nitric oxide signalling [93,106]. Different papers underline the EVs cargo as able to modulate the embryo-mother bidirectional cross-talk [107,108]. In vivo, EVs have been found in uterine fluid and, in vitro, in the culture medium, released from endometrial cancer cell lines (ECC1) [109]. Even human embryos, obtained from In Vitro Fertilization cycles (IVF), can secrete exosomes and microvesicles in the culture medium and it has been demonstrated that embryo EVs are received by primary endometrial cells [110]. Therefore, accurate characterization of their cargo could provide useful information about the quality of the embryo and endometrial receptivity in assisted reproductive medicine; actually, one of the most fascinating challenges in reproductive medicine is the identification of new biomarkers able to indicate the best quality embryos to implant [10]. Currently, Preimplantation Genetic Screening (PGS) is often associated with the morphological evaluation of embryo quality, to detect euploid embryos to implant. Even though the potential of PGS, the invasive removal of cells from the trophectoderm is still considered a critical procedure [111]. For these reasons, the spent culture medium analysis could represent an innovative method to assess embryo competence during implantation [10]. Giacomini et al. in 2017 demonstrated for the first time that human preimplantation embryos at different developmental stages (at day 3 and day 5 after fertilization) can release EVs during their in vitro culture for ART (Assisted Reproductive Technologies) [112]. The authors isolated and characterized the exosomes from embryo-derived conditioned media, according to the guidelines of the MISEV; moreover, the embryo vesicles can be internalized by cultured endometrial cells, suggesting their ability to communicate with the maternal side [112]. Furthermore, co-culture techniques using both embryonic and endometrial cells have been introduced in a paper conducted by Bhadarka et al. [113], with intent to mimic the uterine microenvironment and improve the quality of the embryo; the authors obtained better quality blastocyst with a higher implantation rate, by culturing human embryos coming from women who had undergone Intra Cytoplasmic Sperm Injection (ICSI) with cumulus cells [10,113]. Investigating the nucleic acid constituent of the EVs released by the embryo in the culture media, in 2017 Pallinger et al. demonstrated, by using flow cytometry, that only the embryo releasing a low number of vesicles were competent, probably because a higher presence of nucleic acid could be related to cell injury and, consequently, to embryo damage [114]. Other studies have also shown the presence of miRNAs in the embryo culture medium, correlated with in vitro fertilization methods, embryo aneuploidy and pregnancy outcome [115,116]; Abu-Halima et al. by analyzing extracellular vesicle secretion and miRNA expression in the spent culture media after embryo transfer, observed a reduced miRNA amount, related to decreased EVs secretion from an embryo successfully implanted in comparison with an embryo with a negative outcome [10,117]. Another approach to evaluate embryo-quality is revealed in a study published in 2019 by Battaglia et al., [118]: for the first time, the authors demonstrated the human embryo's ability to secrete exosomes enriched with miRNAs inside the human blastocoel fluid (BF). The bioinformatic and comparative analyses identified the biological function of these miRNAs in critical signalling pathways controlling embryo development, such as pluripotency, cell reprogramming, epigenetic modifications, intercellular communication, cell adhesion and cell fate. MiRNAs of BF reflect the miRNome of embryonic cells and their presence in

exosomes, strongly suggests their important role in mediating cellular signalling among blastocyst cells. Their characterization is important to study the earliest stages of embryogenesis and the complex paths regulating pluripotency. Above all, miRNA expression profiles in BF, could be used as possible minimally invasive biomarkers of embryos, predicting implantation rate in IVF cycles [10,118].

4. Exosomes involved in female pathologies

Several studies proposed exosomes as some of the protagonists involved in a high number of female pathologies - premature ovarian failure (POF), recurrent implantation failure (RIF), preeclampsia, endometriosis, cervical and endometrial cancer, polycystic ovarian syndrome (PCOS) – delivering different cargoes, especially miRNAs which could be regarded as diagnostic biomarkers [43,119–123]. The investigation of regulatory disorders caused by the altered EVs secretion, modifying the regulation of genes and protein expression and several signalling pathways such as wingless signalling pathway (WNT), transforming growth factor beta (TGF- β), neurotrophins, insulin signalling pathways, mitogen-activated protein kinase (MAPK), epidermal growth factor receptor (ErbB) pathways and pathways associated with ubiquitin, could offer insights into implantation failure in women with reproductive diseases [124,125]. For instance, a recent study, conducted by Zhou et al. illustrates for the first time the differential expression patterns of exosomal miRNAs from endometrial stroma cells of women with endometriosis-associated infertility [120]. Hsa-miR-494-3p, hsa-miR-10b-3p, hsa-125b-2-3p, and hsa-miR-1343-3p display higher expression levels and some of their predicted target genes are related to endometrial receptivity: homeobox A10 (HOXA10) and leukemia inhibitory factor (LIF). HOXA10 is involved in morphological arrangements of the uterus and in endometrial regeneration during menstrual cycle [126,127]; instead, LIF is one of the most important cytokines essential for regular implantation [128]. Their expression is significantly decreased in patients with endometriosis, affecting endometrial receptivity and implantation [120]. Another study revealed that circulating exosomes in PCOS follicular fluid had differential miRNAs expression compared to healthy female controls: miR-146a-5p and miR-126-3p expression levels were increased, instead miR-20b-5p, miR-106a-5p, and miR-18a-3p showed a decreasing trend in PCOS patients. The computational analysis highlighted the involvement of miRNAs in MAPK signalling pathway, axon guidance, circadian rhythms, endocytosis, and tumorigenesis circuits suggesting that they may confer a risk of PCOS [129]. Li, H., et al. in a study conducted in 2020, isolated exosomes from women with preeclampsia, reporting that the concentration and the mean diameter of plasma exosomes were greater than healthy controls (women with uncomplicated term pregnancies) [130]. The authors found the up-regulation of miR-153-3p and miR-325-3p into exosomes from preeclampsia [130], which are known to be able to inhibit cell proliferation and promote apoptosis [131]. Recently, researchers have started to investigate the relationship between exosomal miRNAs and their influence on ovarian cancer [123]. Previous studies have revealed that exosomes could influence the chemo-susceptibility in recipient cells by regulating different biological pathways, including cell cycle and apoptosis: for instance, miR-106a, miR-130a, miR-221, miR-222, miR-433, and miR-591 are introduced as modulators of drug resistance in ovarian cancer [132,133]. Additionally, a newer analysis indicated that macrophage-derived exosomes transfer miR-223 to epithelial ovarian cancer cells to promote drug resistance through the PI3K/AKT signalling pathway [123,134]. Besides, Li et al. [135] showed that cancer-associated fibroblasts (CAFs)-derived exosomes induce endometrial cancer progression partially due to the loss of miR-148b in the exosomes, which represent an important tumoral suppressor. MiR-148b targets DNA (cytosine-5) methyltransferase1 (DNMT1) that suppresses endometrial cancer metastasis by increasing epithelial-mesenchymal transition (EMT) [135]. Another study observed that exosomal miR-320a, derived from CAFs, has a lower expression in endometrial cancer cells and tissues: it targets hypoxia-inducible factor 1 subunit alpha (HIF1 α) which reduce the vascular endothelial growth factor a (VEGFA) expression inhibiting cell proliferation [136]. More investigations on the central role of exosomes and their miRNAs cargo in the pathophysiology of reproduction are required to describe the effect of exosomes on the activity

of cancer. A better approaches to learn their activities in the female reproductive system secretome could promote the development of innovative diagnostic and therapeutic tools [18,123].

5. Conclusion and Future Perspectives

This review summarizes the principal implications of exosomal vesicles in a wide range of biological processes related to reproduction, acting as essential mediators of intercellular communication and major regulators of cell functions. The great versatility of exosomes is evident in their involvement in a large number of processes such as germinal cell development and regulation of the reproductive system, tumor growth, tissue homeostasis, immune regulation, disease progression. However, despite the notable progress during the recent years, several challenges remain to be addressed. These include the standardization of isolation and characterization techniques, investigation of specific cargo-loading mechanisms, and optimization of exosome-based therapeutic strategies for clinical translation medicine. Overall, ongoing and future studies on exosomes will be able to underline their full potential in biomedical applications and personalized medicine, to improve diagnosis, treatment and management of diseases, as well as increasing positive outcomes in assisted reproduction techniques.

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