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Extracellular vesicles: Roles in oocytes and emerging therapeutic opportunities

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Abstract

The production of high-quality oocytes requires precisely orchestrated intercellular communication. Extracellular vesicles (EVs) are cell-derived nanoparticles that play a vital role in the transfer of bioactive molecules, which has gained much attention in the field of diagnosis and treatment. Over the past ten years, the participation of EVs in the reproductive processes of oocytes has been broadly studied and has shown great potential for elucidating the intricacies of female reproductive health. This review provides an extensive discussion of the influence of EVs on oocytes, emphasizing their involvement in normal physiology and altered cargo under pathological conditions. In addition, the positive impact of therapeutic EVs on oocyte quality and their role in alleviating ovarian pathological conditions are summarized.

Keywords: Extracellular vesicles; Oocyte; Ovarian diseases

Introduction

The production of functional oocytes is essential to female fertility. However, under pathological conditions such as polycystic ovary syndrome (PCOS) and endometriosis, a reduction in the quality or availability of oocytes with developmental capacity is common, limiting fertilization ability and embryo potential.[1,2] This has prompted extensive research into the underlying mechanisms and effective therapeutic strategies.

Initially recognized as cellular debris,[3] emerging research has highlighted the significance of extracellular vesicles (EVs) in intercellular communication. Based on their biogenesis, EVs are categorized into two major groups: exosomes, which are formed via endosome exocytosis, and microvesicles, which bud directly from the plasma membrane.[4] These biological nanoparticles encapsulate various bioactive molecules (including nucleic acids, proteins, lipids, and metabolites) and traffic between cells locally or systemically. Since 2012 when the presence of exosomes and microvesicles in equine follicular fluid was first reported,[5] the roles of EVs on oocytes have been widely explored. Under normal and pathological conditions, EVs are released and involved in numeric biological processes, as candidate indicators of oocyte quality and follicle health. Moreover, the low toxicity and immunogenicity of EVs make them promising next-generation therapeutic agents, which have demonstrated unequivocally encouraging benefits across various conditions, including cancers, acute myeloid leukemia, and wound healing.[6–8] Furthermore, EV engineering has enabled modifications in biochemical properties for enhanced cargo delivery, paving the way for EV-based therapies.[9]

Despite these advances in knowledge, a detailed review focused exclusively on oocytes remains lacking. This review begins with an overview of the physiological role of EVs in oocyte reproductive processes, followed by a discussion of their negative effects under pathological conditions, focusing on the altered molecular cargo. In addition, we provide a comprehensive update on the

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therapeutic potentials of EVs in improving oocyte quality and alleviating ovarian pathological progression that harms follicle health, followed by innovative precision therapy strategies.

EVs and Oocytes in Reproductive Physiology

The microenvironment responsible for the production of a functional oocyte is composed of theca cells, progressively layered granulosa cells (GCs), and an antrum filled with follicular fluid. Under physiological conditions, EVs intricately orchestrate oocyte reproductive processes [Figure 1].

Early studies analyzing the microRNA (miRNA) profile of intrafollicular exosomes in human and other mammalian samples have revealed multiple targeted pathways, including ubiquitin-mediated signaling which modulates oocyte meiotic resumption; WNT proteins that are expressed at specific stages of follicular development and luteinization; mitogen-activated protein kinase (MAPK) signaling which facilitates GC proliferation and cumulus expansion; members of the transforming growth factor-beta (TGF-β) family which exert permissive effects on MAPK signaling activation in cumulus cells.[10,11] Furthermore, significant correlations have been established between the cargo of EVs and oocyte quality in follicular fluid samples from humans and other mammals [Table 1]. A range of differentially expressed miRNAs has been identified, whose biological functions have been explored in other tissues, primarily in relation to signal transduction, cellular senescence, proliferation, metabolic processing, and cell–cell adhesion.[12–14] Meanwhile, lipid components of follicular fluid EVs can also indicate oocyte quality, possibly working as secondary messengers.[15] In addition, the mRNA levels of mitochondrial electron transport chain genes in follicular fluid exosomes have been reported to positively correlate with follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels in follicular fluid, which are typically elevated in follicles harboring high-quality oocytes.[16] These findings highlight the physiological significance of follicular fluid EVs in the acquisition of oocyte competence.

Besides these observational investigations, in recent years, more experimental evidence has been provided. *In vitro* studies have demonstrated the internalization of follicular fluid-derived EVs into oocytes, GCs, and theca cells, along with improved oocyte development and enhanced functions of somatic cells.[17–19] In mechanism, some encapsulated molecules have been confirmed for their participation in physiological development. Late oocyte maturation requires antioxidative protection.[20] Predicted to target the dedicator of cytokinesis 6, an atypical ornithine nucleotide exchange factor that activates Rho GTPase, exosomal miR-148a-3p released

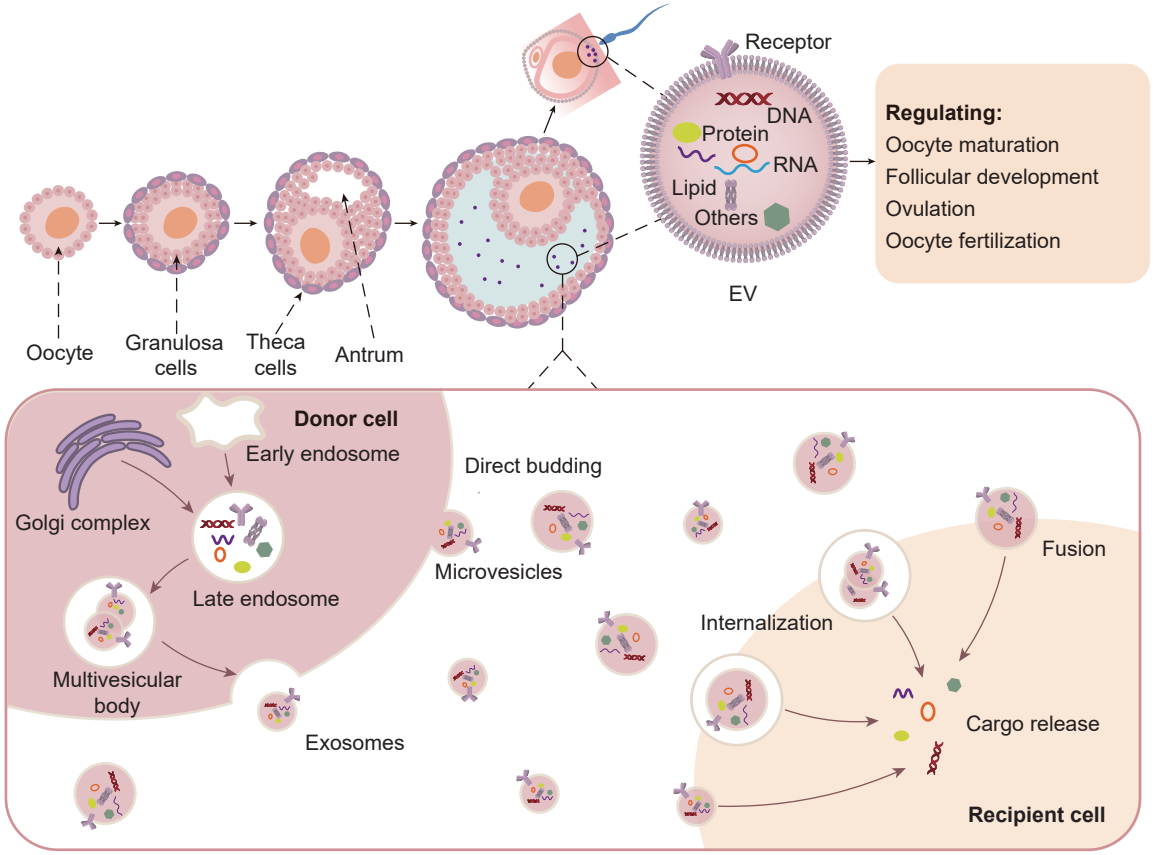

Figure 1: A scheme of EV-mediated intercellular communication in oocyte reproductive processes. EVs are small membrane-bound vesicles containing diverse biomolecules. These vesicles are primarily categorized into exosomes, which are mainly formed through endosomal biogenesis, and microvesicles, which are released via direct budding from the cell plasma membrane. Upon absorption by recipient cells through direct fusion or internalization mechanisms, EVs release cargo molecules that play pivotal roles in oocyte reproductive processes. EVs: Extracellular vesicles.

Table 1: Cargo in the EVs of follicular fluid containing high-quality oocytes.

Species	Upregulated cargo	Downregulated cargo	Oocyte quality criteria	Ref.
Human	miR-214 and miR-454	miR-92a, miR-130b, and miR-888	Fertilization status and embryo quality status	[12]
Human	miR-1246, miR-548ae-5p, miR-505-3p, miR-548t-3p, miR-548au-5p, miR-320e, and miR-1303	miR-513c-5p and miR-548au-3p	Antral follicle count and AMH level	[13]
Porcine	miR-125b, miR-193a-5p, and miR-320	miR-9, miR-206, and miR-6516	Oocyte stain	[14]
Bovine	25 lipids including DAG 36:0+NH4, TAG 56:0+NH4, NAPE 40:1+NH4, etc	–	The capability of the oocyte to develop to the blastocyst stage	[15]
Human	mRNA of ETC complex I subunit 1, ETC complex III cytochrome b	–	Fertilization status and embryo quality status	[16]

AMH: Anti-Mullerian hormone; DAG: Diacylglycerol; ETC: Electron transport chain; EVs: Extracellular vesicles; miR: MicroRNA; NAPE: N-acylphosphatidylethanolamine; Ref.: Reference; TAG: Triacylglycerol; –: Not available.

by GCs improves the antioxidant capacity of porcine oocytes.[21] Moreover, steroidogenesis is important in follicle growth. In this context, exosomal miR-31-5p has been identified to facilitate progesterone synthesis in porcine GCs, via the WNT/B-CATENIN pathway, which is inhibited by secreted frizzled related protein 4 in GCs.[22] Besides, oocyte development and folliculogenesis are highly dependent on glucose metabolism in GCs.[23] By inhibiting b-cell translocation gene 2, miR-21-5p carried by porcine follicular fluid exosomes activates the insulin receptor substrate 1/protein kinase B (AKT) signaling pathway, thus improving glucose uptake in GCs.[18]

Existing evidence also highlights the dynamic coordination of EVs in the differentiation of dominant follicles, preovulatory preparation, and fertilization. Analysis of bovine follicular fluid revealed that EVs from small subordinate follicles are richer in glycerophospholipids and sphingolipids, whereas those from large dominant follicles are enriched in lysophospholipids.[24] These differentially abundant lipid species are involved in processes closely related to oocyte competence and preovulation follicular development, such as the metabolism of linoleic, alpha-linolenic, and arachidonic acids.[24] As the follicle matures, before ovulation, porcine follicular fluid exosomes deliver miR-10b-5p to GCs, targeting brain-derived neurotrophic factor (*BDNF*) mRNA, thus promoting the chemokine secretion, crucial for the recruitment of immune cells and ovulation promotion.[25] Furthermore, during the peri-ovulatory phase, miR-21, miR-132, and miR-212, whose expression appears to be induced by the ovulatory surge of LH, are packaged and released as vesicles in the follicular fluid of mares, targeting genes involved in the expansion of the cumulus-oocyte complex, a critical process for ovulation.[26] Finally, in fertilization, after the sperm cross the zona pellucida, they acquire CD81-containing vesicles released by cumulus cells and located within the zona pellucida.[27] Facilitated by CD81, CD9 released by oocytes is transferred to the sperm as exosomes.[28] This classic exosomal marker is essential for membrane fusion, and its deficiency was reported to result in abnormal microvillar shape and distribution in oocytes.[29] Taken together, these studies further elucidate the communicative role of EVs in temporal reproductive processes.

Altered EVs and Compromised Oocyte Quality under Pathological Conditions

Under ovarian pathological conditions, the normal intercellular communication between oocytes and the surrounding somatic cells is interrupted. Loaded with aberrant molecular cargo, EVs contribute to the deterioration of oocyte quality and follicle health [Table 2].

Altered EVs in PCOS

Attributed to disrupted signaling during folliculogenesis, the oocyte quality of patients with PCOS is often poor, which adversely impacts regular ovulation and embryo quality.[1] Liu *et al*[30] provided direct evidence of the pathological impact of EVs on oocyte quality, as mouse oocytes internalizing follicular fluid EVs from patients with PCOS showed disrupted mitochondrial distribution and spindle function. However, the specific molecular mechanism remains unexplored.

Much attention has been paid to EV-cargo and its pathological effects on the intrafollicular environment, which indirectly jeopardizes oocyte quality. First, they indicate abnormal steroidogenesis patterns. Downregulation of exosomal circular_0008285 was identified in the follicular fluid of patients with PCOS.[31] This molecule acts as a sponge for miR-4644, which inhibits the low-density lipoprotein receptor and interferes with cholesterol transport in GCs.[31] Second, follicular dysplasia due to GC apoptosis contributes to oligo-ovulation. This pathological effect can be exacerbated by the upregulated miR-143-3p in follicular fluid exosomes, which targets bone morphogenetic protein receptor 1A and blocks SMAD1/5/8 signaling, thus accelerating the apoptosis of GCs.[32] In addition, adequate energy supply is crucial for oocyte maturation and folliculogenesis, while the upregulated exosomal miR-143-3p also shows an inhibitory effect on hexokinase 2 to antagonize the glycolysis of GCs in PCOS.[23,33] Recently, another glycolysis-related miRNA, miR-34a-5p, was reported to upregulate in the follicular fluid EVs and specifically bind to the 3′-untranslated region (3′-UTR) of lactate dehydrogenase A mRNA, thus interfering with GC glucose metabolism and leading to apoptosis.[34] Finally, PCOS is an inflammatory condition and EVs can aggravate the intrafollicular pro-inflammatory states. For

Table 2: Alterations in EV-cargo and their impact under pathological conditions.

Pathological conditions	Species	Separation and characterization methods	Altered EV-cargo	Impact (predicted or experimentally validated)	Ref.
PCOS	Human	Ultracentrifugation and filtration; TEM, NTA, and Western blot	circ_0008285↓	Disturbed cholesterol metabolism	[31]
PCOS	Human	exoEasy Maxi Kit (Qiagen); TEM	miR-143-3p↑	Increased apoptosis of GCs	[32]
PCOS	Human	Ultracentrifugation and filtration; TEM, NTA, and Western blot analysis	miR-143-3p↑ miR-155-5p↓	Antagonized glycolysis of GCs; follicular dysplasia	[33]
PCOS	Human	Qiagen exoEasy Maxi kit (Qiagen, Hilden, Germany); TEM, NTA, and Western blot	miR-34a-5p↑	Inhibited glycolysis and increased apoptosis of GCs	[34]
PCOS	Human	ExoQuick-TC Exosome Precipitation Solution Kit (System Biosciences) and filtration; TEM, NTA, and Western blot	S100-A9↑	Exacerbated intrafollicular inflammation	[35]
Ovarian aging	Mare	Ultracentrifugation and Exoquick (System Biosciences); TEM, flow cytometry, and Western blot	miR-181a, miR-375, and miR-513a-3p↑	Oocyte dysmaturity	[5]
Ovarian aging	Human	Ultracentrifugation; TEM	miR-134, miR-190b, and miR-99b-3p↑ miR-21-5p↓	Abnormality in heparan-sulfate biosynthesis, extracellular matrix-receptor interaction, and carbohydrate metabolism; increased apoptosis of GCs	[36]
Ovarian aging	Human	Ultracentrifugation; NTA and Western blot	Progesterone and membrane-associated progesterone receptor↓	Decreased fertilization capacity	[38]
Endometriosis	Mice	Ultracentrifugation and filtration; TEM, NTA, and Western blot	miR-1966-5p, miR-690, let-7c-1-3p, miR-23b-3p, miR-23a-3p, miR-221-3p, miR-140-5p, miR-369-5p, and miR-26b-5p↑ miR-5106, miR-5119, miR-485-5p, miR-342-5p, and miR-5128↓	Oocyte dysmaturity	[40]
Endometriosis	Human	Ultracentrifugation and filtration; TEM, NTA, and Western blot	miR-122-5p↑	Perturbed glucose metabolism in GCs	[41]
POI	Mice	Ultracentrifugation and filtration; TEM, and Western blot	miR-122-5p↑	Increased apoptosis of GCs	[43]
POF	Human	Centrifugation and filtration; TEM, and Western blot	miR-19b-3p↑	Disturbed oocyte maturation and ovarian steroidogenesis; increased apoptosis of GCs	[44]
POF	Rabbit	Exosome isolation kit (Gibco®, USA); TEM, and Western blot	miR-10a-5p↑	Increased apoptosis and senescence of GCs; inhibited GC proliferation	[45]
EDCs	Human	Ultracentrifugation; TEM, NTA, and Western blot	miR-116-5p↑	Impaired oocyte development	[48]
EDCs	Human	Ultracentrifugation; NTA and flow cytometry	miR-27b-3p↓	Increased apoptosis of GCs; oocyte dysmaturity	[49]

circ_0008285: Circular_0008285; EDCs: Endocrine-disrupting chemicals; EV: Extracellular vesicle; GCs: Granulosa cells; miR: MicroRNA; NTA: Nanoparticle tracking analysis; PCOS: Polycystic ovary syndrome; POI: Primary ovarian insufficiency; POF: Premature ovarian failure; Ref: Reference; S100-A9: S100 calcium-binding protein A9; TEM: Transmission electron microscopy.

example, S100 calcium-binding protein A9 transported by follicular fluid exosomes activates the nuclear factor kappa B (NF-κB) signaling pathway, promoting a persistent inflammatory response in a positive feedback loop.[35] These findings not only elucidate the molecular mechanisms underlying impaired oocyte quality in patients with PCOS but also provide novel insights for PCOS diagnosis.

Altered EVs in ovarian aging

Reduced oocyte quality and the associated failure of fertilization greatly contribute to the infertility problem in aged women. da Silveira *et al*[5] first identified age-related miRNA differences in follicular fluid EVs from mares. Subsequently, differences in miRNA profile were detected

in human follicular fluid microvesicles. According to Diez-Fraile *et al*,[36] with age, the upregulated miR-134 was predicted to target *BCL2*, a known inhibitor of apoptosis; the downregulated miR-21-5p was predicted to target the p53 and TGF-β pathways, thus leading to defects in folliculogenesis. Notably, this seems to be a positive feedback, as p53 pathway activation further increases the release of small CD81-containing EVs, which transmit apoptotic signals from GCs.[37] Besides alterations in the miRNA profile, reduced levels of progesterone and its receptor in follicular fluid EVs are also prominent with age, which seems to correlate with diminished fertilization capacity, the possible mechanism may relate to the release of sperm from the isthmus of the oviduct.[38] These studies highlight the age-related variations in EVs. However, it is important to note that these investigations are observational, lacking experimental evidence of the impact of altered EV-cargo. Future *in vivo* and *in vitro* studies are needed to further validate their pathological effects.

Altered EVs in endometriosis

Endometriosis is a fertility-impairing condition attributed to the implantation of endometrial cells outside the endometrium. On the one hand, pelvic adhesions and chronic inflammation may impede ovulation, fertilization, and embryo implantation; on the other hand, the decreased number of mature oocytes retrieved in patients with endometriosis indicates compromised oocyte quality.[2] Iron overload caused by retrograde menstruation and periodic hemorrhage from ectopic lesions is a crucial pathological mechanism of endometriosis and can impede oocyte development.[39] Further investigation revealed that GCs in iron overload release abnormal exosomes to oocytes, which disturb key signaling pathways, including calcium, MAPK, cell cycle regulation, oocyte meiosis, and the ferroptosis pathway, thereby impeding oocyte maturation.[40] In addition, oocyte energy supply largely depends on cumulus cells' pyruvate metabolism, which is prominently disturbed in endometriosis.[23] Recently, overexpression of miR-122-5p in follicular fluid exosomes from untreated endometriosis patients was observed, inhibiting aldolase A, a crucial enzyme in glucose metabolism, leading to inadequate oocyte energy supply and reduced quality.[41] These findings shed light on the mechanism of dysmaturity of oocytes in patients with endometriosis.

Altered EVs in primary ovarian insufficiency and premature ovarian failure

Despite their different clinical definitions, premature ovarian failure (POF) and primary ovarian insufficiency (POI) are both characterized by decreased follicle reserves and a marked reduction in functional oocytes.[42] In cyclophosphamide-induced mouse models of POI, the exosomal miR-122-5p level was significantly elevated in ovarian tissues, promoting GC apoptosis by suppressing the expression of *Bcl9*.[43] Apart from ovarian-derived EVs, EVs derived from plasma, which can cross the blood-follicle barrier, have also shown pathological effects. Analysis of differentially expressed miRNAs in plasma exosomes from patients with POF and healthy controls revealed the suppression of pathways involved in oocyte meiosis and cell proliferation.[44] Among them, upregulated miR-19b-3p is a key pathological factor, by inhibiting bone morphogenetic protein receptor 2 transcriptional activity, which is essential in oocyte maturation.[44] Recently, in the plasma exosomes of POF rabbits, high expression of miR-10a-5p was found to target *BDNF* mRNA, thereby inhibiting the AKT/mammalian target of rapamycin (mTOR) pathway in GCs, leading to increased apoptosis.[45] Notably, exosomal miR-10b-5p, another member of the highly conserved miR-10 family, has been reported to promote physiological ovulation-related chemokine secretion of GCs, also through the inhibition of *BDNF*.[25] The mechanism by which these molecules coordinate the fate and activity of GCs remains to be explored.

Altered EVs in environment-related infertility

The widespread use of synthetic chemicals in industry and agriculture leads to the accumulation of various chemicals in the body. These chemicals, known as endocrine-disrupting chemicals (EDCs), disrupt hormonal homeostasis.[46] In recent years, growing concerns have emerged regarding the impact of EDCs on the dysfunction of the female reproductive system. Phthalates are a ubiquitous class of EDCs that interfere with sex hormone levels in females.[47] Elevated monobutyl phthalate levels were detected in female follicular fluid, significantly upregulating miR-116-5p in EVs, which impairs oocyte maturation by targeting forkhead box O3a, a crucial factor in antioxidative stress.[48] Bisphenol A is another widely investigated EDC that has gained attention for its estrogen-like effects and gonadal toxicity. In follicular fluid, supraphysiological concentration of bisphenol A results in miR-27b-3p downregulation in EVs, disrupting fas-associated death domain protein inhibition, promoting GC apoptosis, and subsequently impairing oocyte quality.[49] These findings further underscore the importance of avoiding potential exposure to these chemicals.

Taken together, the contents of EVs undergo alterations under pathological conditions, leading to impaired oocyte quality and follicle health [Figure 2]. However, although the list of aberrant molecules carried by EVs is large, not all have been experimentally confirmed for their pathological impact. Some conclusions were drawn from the prediction of targets or the results in other tissues. To fulfill the diagnostic potential of providing first-hand information on oocyte quality, future *in vitro* and *in vivo* studies are required to carefully validate the impact of altered EV-cargo. In addition, as the immediate environment for oocyte development, many studies that investigate the pathological role of EVs on oocytes focused on those derived from follicular fluid. However, follicular fluid accessed in clinical settings or from small mammals is limited in volume, which necessitates exploring effective separation and characterization methods. Existing studies show that many researchers prefer combining methods [Table 2]. Recent advancements in detection techniques are noteworthy. A study developed a silicon-based sensor platform that exhibited high efficiency in separating, concentrating, and quantifying follicular fluid small EVs.[50] It is envisioned that the integration of medicine

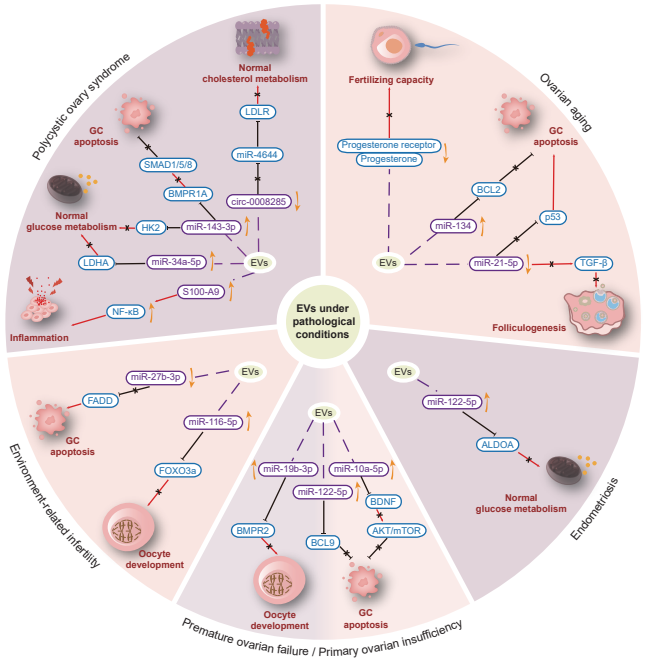

Figure 2: Mechanistic insights into the impact of EVs under ovarian pathological conditions. EVs exert pathological effects in conditions such as polycystic ovary syndrome, ovarian aging, endometriosis, primary ovarian insufficiency, premature ovarian failure, and environment-related infertility by transporting essential cargo vital for follicle health. The cargo is shown in boxes with purple margins and their downstream targets are indicated by boxes with blue margins. AKT: Protein kinase B; ALDOA: Aldolase A; BDNF: Brain-derived neurotrophic factor; BMPR1A: Bone morphogenetic protein receptor 1A; BMPR2: Bone morphogenetic protein receptor 2; circ_0008285: Circular_0008285; EVs: Extracellular vesicles; FADD: Fas-associated death domain protein; FOXO3a: Forkhead box O3a; GC: Granulosa cell; HK2: Hexokinase 2; LDHA: Lactate dehydrogenase A; LDLR: Low-density lipoprotein receptor; miR: MicroRNA; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa B; S100-A9: S100 calcium-binding protein A9; TGF-β: Transforming growth factor-beta.

and engineering will enable continued advancements in efficient and convenient detection techniques.

Therapeutic Potential of EVs in Ovarian Pathological Progression

The favorable properties of EVs position them as promising candidates for treating diseases. First, the unique bilayer membrane structure and its surface charge confer stability in the transport of bioactive cargo.[51] Second, as non-living organisms originating from mammalian cells, EVs are biocompatible and can be immunologically inert because they can be sourced from the patients themselves.[4] Furthermore, EVs have already demonstrated favorable outcomes in the clinical trials of some diseases.[6] Here, we focused on how EVs from various sources protect oocytes and follicles under ovarian pathological conditions.

Improving the quality of oocytes

The number of mammalian oocytes is fixed before birth and gradually diminishes owing to natural aging and some ovarian pathological conditions. Recent studies have highlighted the potential of EVs in safeguarding the quality of this limited oocyte pool. Yang *et al*[52] reported that human umbilical cord mesenchymal stem cell-derived exosomes (hucMSCs-Exos) can accumulate in primordial oocytes and carry functional molecules such as miR-146a-5p and miR-21-5p, which upregulate phosphoinositide 3-kinase (PI3K)/mTOR signaling pathway and activate primordial follicles. In aged mice, intraovarian injection of these therapeutic exosomes reduced reactive oxygen species levels and decreased the percentage of oocytes exhibiting abnormal spindle morphology.[52] Similar improvement in oocyte quality was also observed in aged oocytes of *in vitro* maturation.[53]

Mechanistically, therapeutic EVs can improve oocyte mitochondrial function, which is crucial for oocyte viability. After administrating exosomes derived from human amniotic mesenchymal stem cells, the exosomal miR-320a inhibits the pathologically elevated Sirtuin signaling in oocytes, whose downstream target, GTPase optic atrophy type 1, acts as a central cellular energy sensor that participates in mitochondrial fusion.[54] Furthermore, therapeutic EVs can transfer specific regulatory proteins that help oocyte spindle stabilization. Adding follicular fluid EVs to the medium of vitrified cat oocytes during thawing has been reported to enhance meiotic resumption because these vesicles deliver essential structural and functional proteins such as actin-related protein 2/3 complex, myosin, and F-actin.[55] This is implicative in assisted reproductive technology, as the efficacy of vitrification in immature oocytes is still suboptimal due to compromised meiotic competence.[56]

Aiding in the survival of GCs

Oocytes lack certain metabolic processes essential for their development and GCs can compensate for the deficiency.[57] Moreover, GCs shape the hormonal microenvironment which determines oocyte competence and fertilization ability.[58] However, under pathological conditions, the survival of GCs is threatened and accompanied by their dysfunction. Therefore, protecting GCs is essential for restoring oocyte quality and ovarian function.

Some therapeutic EVs can ideally meet this requirement and the molecular mechanisms have been extensively explored. The dysregulation of the PI3K/AKT/p53 pathway is detrimental to GC survival. MiR-144-5p, delivered by exosomes derived from bone marrow mesenchymal stem cells (BMSCs-Exos), targets the phosphatase and tensin homolog, a negative regulator of integrin-linked kinase, leading to the phosphorylation of AKT and ultimately mitigating the progression of chemotherapy-induced ovarian failure.[59] Similarly, by increasing the phosphorylation of SMAD-3, exosomal thrombospondin-1 transported by menstrual blood stromal cells upregulates the SMAD/PI3K/AKT/p53 signaling pathway and inhibits GC apoptosis.[60] Moreover, p53 is also directly inhibited by the therapeutic EVs. MiR-664-5p delivered by BMSCs-Exos can bind to the 3′-UTR of *p53* mRNA, thereby protecting GCs from cisplatin-induced apoptosis.[61] Meanwhile, the activating transcription factor 4/activating transcription factor 3/C/EBP homologous protein (ATF4/ATF3/CHOP) signaling pathway, which is broadly involved in apoptosis-related diseases, is also one of the targets. By downregulating the Kruppel-like factor 6, miR-22-3p

carried by hucMSCs-Exos can inhibit the ATF4/ATF3/CHOP pathway, thereby alleviating GC apoptosis in the POF model.[62] In addition, recent research demonstrated that BMSCs-Exos loaded with miR-21-5p targets msh homeobox 1 and activates the Notch signaling pathway, thus improving ovarian function.[63] The Hippo pathway, critical in GC proliferation and follicle activation, is also one of the activated targets of therapeutic EVs, through the inhibition of mammalian Ste20-like kinases 1/2 and promoting the nuclear accumulation of yes-associated protein (YAP).[64] Besides, chronic GC inflammation characterizes ovarian pathologies such as PCOS and POI. By inhibiting the phosphorylation of IκB and p65 as well as the nuclear translocation of p65, hucMSCs-Exos can inhibit the NF-κB pathway and reduce the expression of inflammatory factors in GCs from patients with PCOS.[65] According to Xie *et al*,[66] BMSCs-Exos is also able to inhibit NF-κB while downregulating the NOD-like receptor protein 3-mediated pyroptosis pathway in GCs from patients with autoimmune POI. Taken together, these studies fully revealed the potential of therapeutic EVs in reversing the fate of GCs under pathological conditions.

Hormone secretion reflects the viability of GCs. Several studies have documented the restoration of normal hormone levels in ovarian insufficiency models following the administration of therapeutic EVs, including decreased levels of FSH and LH and increased levels of anti-Mullerian hormone and estradiol.[59,66] This is partly attributed to their effect in aiding GC survival, thus increasing the number of healthy follicles and restoring the estrous cycle. In addition, therapeutic EVs also deliver specific cargo that regulates hormone synthesis pathways. MiR-21 may be one of the candidates. Transported by hucMSCs-Exos, miR-21 inhibited the expression of large tumor suppressor 1, a serine/threonine kinase, and subsequently reduced the phosphorylation of lysyl oxidase-like 2 and YAP, thus promoting estrogen secretion in GCs.[67] The effect is not limited to hucMSCs-Exos, as miR-21 has also been reported to be present in BMSCs-Exos and increase the expression of the hormone synthesis-related genes in autoimmune POI mice.[63]

Regulating ovarian angiogenesis

Supplying oxygen, gonadotrophins, and critical nutrients such as steroid precursors, the ovarian vascular network is of great importance in producing high-competence oocytes.[68] Abnormal vasculature characterizes numerous ovarian pathologies, while therapeutic EVs regulate angiogenesis to align it with the physiological needs of a healthy ovary.

Under conditions such as POF and POI, downregulated angiogenic pathways and decreased vascular stability are prominent.[68] Qu *et al*[69] reported that hucMSCs-Exos carry miR-126-3p, a dominant molecule for angiogenesis and vascular stability. By binding to the 3′-UTR sequence, miR-126-3p downregulates the expression of phosphoinositide-3-kinase regulatory subunit 2 and activates the PI3K/AKT/mTOR signaling pathway, thereby upregulating angiogenesis-related cytokines, including vascular endothelial growth factor (VEGF), insulin-like growth factor-1, and angiogenin.[69] Poor coverage of α-SMA positive cells marks poor vessel maturation. Histologically, the administration of EVs derived from mesenchymal stem cells (MSCs-EVs) normalized the distribution of α-SMA positive cells in stromal and follicle-associated vessels in the chemotherapy-induced POF model, further validating the restoration of follicular blood supply.[70]

However, in endometriosis, angiogenesis is hyperactivated, which is necessary for the development and sustenance of endometriotic lesions.[71] As mentioned above, iron overload caused by periodic hemorrhage from ectopic lesions harms oocyte quality.[39] In patients with endometriosis, M2 macrophages are selectively activated, promoting angiogenesis and fibrosis.[72] Adding nanovesicles from M1 macrophages was reported to reprogram the M2 macrophages to M1 macrophages and curtailed the proangiogenic effects.[72] In addition, endometriotic cells treated with exosomes derived from menstrual blood-derived mesenchymal stem cells exhibit reduced VEGF expression.[73] Ovarian angiogenesis is also dysregulated in PCOS. The superficial ovarian cortex vascularization in patients with PCOS has been reported to exhibit a two-fold increase compared to age-matched controls and to be inversely related to the follicle reserve.[74] A recent study reported that in mouse models of PCOS, the administration of BMSCs-Exos significantly reduced endothelial expression of platelet endothelial cell adhesion molecule-1, a key regulator in pathological blood vessel formation and maintenance.[75] However, the specific EV-components that regulate angiogenesis require further investigation, because the careful selection of EV-cargo based on pathological conditions (either upregulated or downregulated angiogenesis) is essential for practical applications.

Inhibiting ovarian fibrosis

Ovarian fibrosis has been gaining increasing attention these years. Increased stromal collagen deposition and reduced hyaluronan matrices in the ovary form a rigid barrier that constrains follicle growth and prevents ovulation.[76] Therapeutic EVs can ameliorate the detrimental situation by targeting key factors involved in fibrosis. TGF-β signaling pathway is a classic fibrosis pathway through which fibroblast growth and collagen formation are regulated.[77] Amniotic fluid-derived exosomes have been reported to activate SMAD-6, an inhibitor of TGF-β signaling, and decrease collagen density of the ovaries of POI rats.[78] Downstream of TGF-β, connective tissue growth factor (CTGF) is another target for anti-fibrosis therapy.[77] A liver fibrosis study identified a promising therapeutic target: miR-214 can bind directly to the 3′-UTR of *CTGF* mRNA to inhibit its expression.[79] In endometrial tissues of patients with endometriosis, reduced expression of miR-214 is associated with increased fibrosis at ectopic sites.[80] Notably, exosomes serve as critical vehicles for the intercellular transfer of this miRNA in endometriosis.[81] Transfecting miR-214 into endometrial stromal cells, the secreted exosomes significantly alleviated fibrosis progression in endometriosis mice.[80] These findings provide insights

for addressing follicle loss and ovulation issues associated with rigid ovarian structures.

Collectively, therapeutic EVs can ameliorate the pathological progression of ovarian diseases, thereby supporting the normal reproductive processes of oocytes within the ovary [Figure 3]. Notably, instead of rescuing the already compromised fertility, pre-treatment with therapeutic EVs can prevent the pathological outcomes in advance. A recent study reported that intraovarian injection of MSCs-Exos before chemotherapy can induce the expression of several ATP synthase-binding cassette transporter proteins in GCs, which play key roles in drug efflux and mitochondrial protection against oxidative stress.[82] This has special implications for preventing chemotherapy-associated gonadal toxicity.

However, although the therapeutic effects of EVs have been broadly validated, their widespread application is still hindered by inadequate ovarian targeting and poor local retention, which necessitates exploring the pre-conditioning strategies to enhance the efficacy. Genetic engineering is one of the main strategies for enhancing the targeting ability of EVs by adding fusion proteins to their membrane surface.[9] The membrane protein lysosome-associated membrane glycoprotein 2b (LAMP2b) is commonly targeted in engineering. Alharbi *et al*[83] engineered LAMP2b with an ephrin-B2 ligand and reported significantly enhanced targeting efficiency toward ovarian cancer cells. However, attempts to engineer EVs for the treatment of noncancerous ovarian diseases remain blank. Several studies have also explored effective administrating methods to improve EV delivery. In addition to replacing intravenous injections with local injections, Xin *et al*[84] combined exosomes isolated from umbilical cord mesenchymal stem cells (ucMSCs) with collagen scaffolds, thereby improving local retention of EVs in the uterus. Furthermore, Jiao *et al*[85] practiced a combination of ucMSCs and auto-crosslinked hyaluronic acid gel in the ovary and verified their effects on follicle survival. Given recent

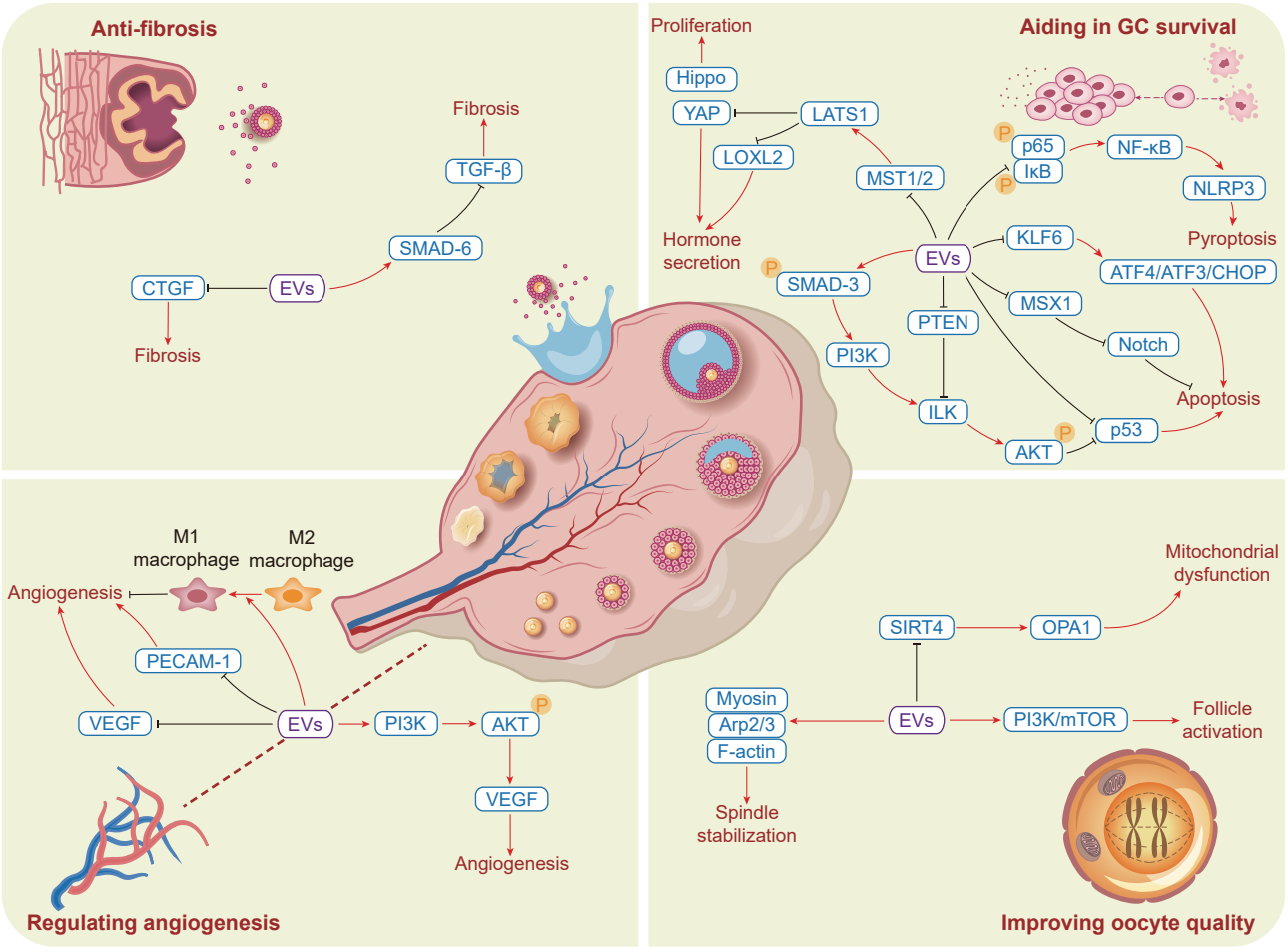

Figure 3: EVs as therapeutic agents in ovarian pathological conditions. First, EVs can improve oocyte quality by alleviating mitochondrial dysfunction and stabilizing spindle formation. Additionally, through anti-apoptotic and anti-pyroptotic mechanisms, EVs exhibit potent effects in enhancing GC viability and functions. They also play a crucial role in promoting GC proliferation and hormone secretion. Moreover, EVs regulate ovarian angiogenesis to align it with the physiological demands of a healthy ovary. Finally, EVs demonstrate promising therapeutic potential in preventing ovarian fibrosis, which could otherwise impair folliculogenesis and ovulation. AKT: Protein kinase B; Arp2/3: Actin-related protein 2/3 complex; ATF3: Activating transcription factor 3; ATF4: Activating transcription factor 4; CHOP: C/EBP homologous protein; CTGF: Connective tissue growth factor; EVs: Extracellular vesicles; GC: Granulosa cell; ILK: Integrin-linked kinase; KLF6: Kruppel-like factor 6; LATS1: Large tumor suppressor 1; LOXL2: Lysyl oxidase-like 2; MST1/2: Mammalian Ste20-like kinases 1/2; MSX1: Msh homeobox 1; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa B; NLRP3: NOD-like receptor protein 3; OPA1: Optic atrophy type 1; PECAM-1: Platelet endothelial cell adhesion molecule-1; PI3K: Phosphoinositide 3-kinase; PTEN: Phosphatase and tensin homolog; SIRT4: Sirtuin 4; TGF-β: Transforming growth factor-beta; VEGF: Vascular endothelial growth factor; YAP: Yes-associated protein.

advances in biomaterials, therapeutic EVs incorporated into bioengineering materials may represent a promising delivery strategy.

Conclusions and Perspectives

The first publication on EVs in the 1980s marked the beginning of a rapidly expanding scientific field. In this article, we review the involvement of EVs in the dynamic orchestration of oocyte reproductive processes from a physiological perspective, as well as the detrimental effects of their abnormalities under pathological conditions. In recent years, investigations on the role of EVs in oocytes have undergone a transition from descriptive to experimental validation, which focuses more on the encapsulated molecular mediators and on providing experimental evidence of their functions. This significantly contributes to the understanding of intercellular communications in producing high-competence oocytes. Given the fascinating natural properties and engineering futures of EVs, we also highlight the therapeutic potential of EVs in reversing ovarian pathological progression, which is closely associated with the fate of oocytes and follicles. However, their applications are still confined to animal models and early-stage clinical trials. In the future, a continuous in-depth exploration of engineered EVs will facilitate the earlier realization of their translation from bench to bedside.

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Conflicts of interest

None.

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