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The effect of exosomes on oocyte maturation

Eksozomların oosit maturasyonuna etkisi

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ABSTRACT

Aim: Numerous infertile patients face challenges in oocyte maturation during in vitro fertilization treatment. Hormonal dysregulation, mitochondrial dysfunction, abnormal organelle distribution within the ooplasm, and biological, genetic, and epigenetic factors lead to oocyte maturation arrest. Oocyte maturation involves the secretion of extracellular vesicles, known as exosomes, by surrounding granulosa cells into the follicular fluid. This review examines the mechanisms by which exosomes influence oocyte maturation, evaluates their effects on oocyte maturation in diverse female infertile patient groups, discusses the therapeutic potential of exosomes in oocyte maturation.

Materials and Methods: Studies published up to September 2024 were collected from the PubMed database. The analysis methodology included the following keywords: exosome or extracellular vesicles or exosomes in reproductive medicine and oocyte maturation, diminished ovarian reserve, polycystic ovary syndrome, premature ovarian insufficiency and therapeutic potential of exosomes. This review focused on studies about exosomes in oocyte maturation and female infertility. The inclusion criteria for the studies were: studies involving patients diagnosed with (1) Premature ovarian insufficiency, (2) Diminished ovarian reserve, or (3) Polycystic ovary syndrome. Male factor infertility, tubal factor infertility, and endometriosis were excluded.

Results: Existing literature demonstrates that exosomes exert crucial effects and a regulatory role on oocyte maturation. Exosomes modulate the processes of ovarian granulosa and cumulus cells to affect follicular development.

Conclusion: The function of exosomes in oocyte maturation may be further clarified through detailed analysis of their specific proteins and therapeutic potential as a nascent alternative treatment for infertility, particularly in patients with diminished ovarian reserve.

Keywords: Extracellular Vesicle, Exosome, Oocyte Maturation, Diminished Ovarian Reserve, Polycystic Ovarian Syndrome

ÖZ

Amaç: *Çok sayıda infertil hasta, in vitro fertilizasyon tedavisi sırasında oosit olgunlaşma sürecinde zorluk yaşamaktadır. Hormonal düzensizlik, mitokondriyal disfonksiyon, ooplazma içinde anormal organel dağılımı, biyolojik, genetik ve epigenetik faktörler oosit maturasyonunun durmasına neden olmaktadır. Oosit maturasyonu sürecinde etrafını çevreleyen granüloza hücrelerinden eksozom olarak bilinen ekstraselüler veziküllerin folikül sıvısına salınımı gerçekleşmektedir. Bu derlemede oosit maturasyonunu eksozomların hangi mekanizmalarla etkilediği incelenmekte, çeşitli kadın infertil hasta gruplarında oosit maturasyonu üzerindeki etkileri değerlendirilmekte ve eksozomların oosit maturasyonundaki terapötik potansiyelleri ortaya konulmaktadır.*

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Gereç ve Yöntem: *Eylül 2024'e kadar yayınlanmış çalışmalar PubMed veri tabanından toplanmıştır. Arama stratejisi aşağıdaki anahtar kelimeleri içermiştir: eksozom veya ekstraselüler veziküller veya üreme tıbbında eksozomlar ve oosit olgunlaşması ve azalmış over rezervi ve polikistik over sendromu ve erken over yetmezliği ve eksozomların terapötik potansiyeli. Bu derleme, eksozomların kadın faktörüne bağlı infertilitede oosit olgunlaşmasındaki rolünü araştıran çalışmalara odaklanmıştır. Çalışmalar için dâhil edilme kriterleri: (1) Erken over yetmezliği, (2) Azalmış over rezervi veya (3) Polikistik over sendromu tanısı almış hastaları içeren çalışmalar olmuştur. Erkek faktörüne bağlı infertilite, tubal faktöre bağlı infertilite ve endometriozis üzerine odaklanan çalışmalar hariç tutulmuştur.*

Bulgular*: Mevcut literatür incelememiz, eksozomların oosit maturasyonu üzerinde önemli etkileri olduğunu ve düzenleyici bir rol oynadığını ortaya koymaktadır. Kanıtlar, eksozomların foliküler gelişimi etkilemek için ovaryan granüloza ve kumulus hücrelerinin fonksiyonlarını düzenlediğini göstermektedir.*

Sonuç: *Eksozomların spesifik proteinlerinin ayrıntılı analizi ile oosit maturasyonundaki rolü daha detaylı aydınlatılabilir. Özellikle azalmış over rezervine sahip hastalarda infertilite tedavisinde gelecekte alternatif bir terapötik potansiyele sahip olduklarını düşündürmektedir.*

Anahtar Sözcükler: *Ekstraselüler Vezikül, Eksozom, Oosit Maturasyonu, Azalmış Over Rezervi, Polikistik Over Sendromu*

INTRODUCTION

The term extracellular vesicle (EV) defines a diverse group of cell-released particles delimited by a lipid bilayer and characterized by an inability to self-replicate and the absence of a functional nucleus. Due to their complex molecular composition, EVs may reveal the physiological state of their cellular origin and are capable of eliciting alterations in the roles and characteristics of recipient cells. EVs are increasingly recognized as potent biomarkers fueled by the escalating interest in exosomes. Further evidence of this growing scientific focus is the consistent year-over-year increase in the number of research investigations dedicated to exosomes (1).

In 2024 Welsh et al. and in 2016 Tkach et al. indicated that extracellular vesicles (EVs), released by cells, have diverse features and functions in many biological processes, which depend on their complex contents. All cell types release EVs, ranging in size from nanometers to micrometers. Their varied structure and molecular composition allow for significant biological discoveries, but further research is needed (1,2).

In 2012, Kalra et al., classified the extracellular vesicles by subdividing them into three main classes based on biogenesis. Table-1 summarizes Kalra's classifications.

In 2013, El Andaloussi et al. alternatively classified extracellular vesicles categorized by their cellular source and/or biological role as described in Table-2.

Table-1. Extracellular Vesicle Classification Based on Biogenesis

Vesicle Type	Biogenesis Mechanism / Origin
Exosome	40-100 nm endocytic vesicles (4).
Ectosome or shedding Microvesicles	50-1,000 nm vesicles formed by outward plasma membrane budding (4).
Apoptotic Body	50-5,000 nm vesicles are classified as apoptotic bodies, originating from cells undergoing programmed cell death (4)

Table-2. Classification Based on Cellular Origin/Biological Function

Vesicle Type	Cellular Origin / Function
Ectosomes	Neutrophils or monocytes / Vesicles involved in immune responses and inflammation (5).
Microparticles	Platelets or endothelial cells (from blood) / Involved in blood coagulation and vascular functions (5).
Tolerosomes	Serum of antigen-administered mice /Associated with immune tolerance (5)
Prostatosomes	Seminal fluid / Related to sperm function and reproductive processes (5).
Cardiosomes	Cardiomyocytes / Involved in heart cell communication and repair (5).
Vexosomes	Adeno-associated virus vectors / Used in gene therapy; carry viral vector components (5).

One of the most comprehensive classifications and categorizations of extracellular vesicles, encompassing separation, characterization, engineering, and clinical applications as defined by Welsh et al. in 2024, is presented within the 'Minimum Information for Studies of Extracellular Vesicles' (MISEV) guidelines, which aim to assist all professionals of EV investigation and implementation, as described in (Table-3).

Table-3. ISEV (International Society for Extracellular Vesicles) Nomenclature

Term	Definition/Description
Extracellular Vesicle (EV)	EVs are lipid bilayer-enclosed particles that cannot replicate and lack a functional nucleus (1).
Non-vesicular Extracellular Particle	Multimolecular extracellular particles not surrounded by a lipid bilayer (1).
Extracellular Particle	General term for both vesicular and non-vesicular particles outside the cell (1).
Extracellular Vesicle Mimetic	Artificially produced EV-like particles (1).
Synthetic Vesicles	Hybrid structures reconstructed from molecular components or fusion of liposomes and natural EVs (1).
Small EVs (Operational Term)	Vesicles typically ≤ 200 nm in diameter; actual size depends on the measurement method (1).
Large EVs (Operational Term)	Vesicles typically > 200 nm in diameter; size varies depending on measurement method (1).
Other Operational Terms	Classification by physical properties (size, density), and protein content, cellular origin, or production conditions (1).
Exosome (Specific Term)	Refers to EVs of endosomal origin; not to be used unless subcellular origin is confirmed (1).
Ectosome	Originates from the plasma membrane; overlaps with exosome sizes but has broader diameter range (1).
Exosome-like Vesicle	Term referring to vesicles of endosomal origin (1)
Microvesicle (MV)	Historically used for large EVs or all EVs; may cause confusion due to non-specific usage (1).

The exosome membrane is mainly composed of lipids, including sphingolipids, ceramides, cholesterol, and diacylglycerol, a feature that differentiates it from other extracellular vesicles (6). Microvesicles, lipid bilayer spheres from the plasma membrane, originate either from direct cell membrane budding (ectosomes) or the endolysosomal pathway (exosomes) (7). Despite their high phosphatidylserine content, microvesicle membranes more closely resemble the parent cell membrane (8).

Biology and Biogenesis of Exosomes

Exosomes are defined as extracellular vesicles, which exhibit a lipid bilayer membrane composition with a relatively uniform size, characterized by diameters varying from 30 to 150 nm. Exosomes are vesicles that encapsulate a diverse array of biologically active molecules, such as cell-specific RNA, DNA, lipids, and proteins, thereby facilitating the transfer of genetic material to target cells and consequently affect normal cellular functions within those cells (9,10). Investigations have revealed the presence of exosomes across a diverse array of biological matrices, including plasma, urine, semen, saliva, bronchial fluid, cerebral spinal fluid, breast milk, serum, amniotic fluid, synovial fluid, follicular fluid, lymph, bile, gastric acid, and tears (6,11,12).

Detailed studies have determined that exosomes are endocytic derived vesicles secreted by numerous cells (13–16). Supporting that exosomes arise from inward budding of the early endosome's limiting membrane as it matures into multivesicular bodies (MVB) (7,17,18). MVBs are degraded by lysosomes, or they fuse with the cell's plasma membrane, expelling their contents, including exosomes, into the extracellular environment (19). In 2023, Chen et al., and in 2010, Wollert et al., stated that the MVB regulation process is carried out through endosomal sorting complexes required for transport called the exosome formation and release (ESCRT) pathway (20,21). The outline of exosome biogenesis is shown in (Figure-1).

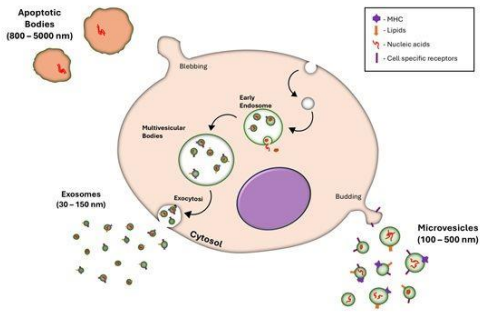

Figure-1. Diagrammatic illustration of exosome biogenesis (Created by Hakan Darici)

Exosomes in intercellular communication

In 2014 Colombo et al., and in 2019, Doyle et al., demonstrated that extracellular vesicles are significant mediators of intercellular communication (6,14). Exosomal membrane molecules, such as integrins, facilitate the targeting of recipient cells (22).

In 2010, Ashiru et al., indicated that exosomes are internalized by target cells through multiple pathways. Exosome membrane protein interactions with classical receptor ligands can alter target cell function (23). In 2014, Mulcahy et al., described other methods of exosome uptake include phagocytosis, clathrin-mediated endocytosis, caveolin-dependent endocytosis, micropinocytosis, and lipid bridging (24). In 2012, Montecalvo et al., demonstrated that a mechanism for signal transduction involves the direct contact of the exosome membrane with the target cell membrane (25).

In 2019, Nair et al., have posited that exosomal proteins modulate signaling pathways by modifying phosphorylation and transferring genetic material. Research further indicates that microvesicles encapsulate proteins, microRNAs (miRNAs), messenger RNAs (mRNAs), and small DNA fragments (17). In 2018, Yu et al., also suggest that exosomal miRNAs can modulate the expression of specific target proteins or induce phenotypic alterations in recipient cells (26).

By virtue of their recognized roles in intercellular communication mechanisms, exosomes present themselves as promising advanced investigation platforms for targeted drug and gene delivery. Their intrinsic stability, minimal immunogenicity, and substantial tissue and cell penetration capabilities represent critical unique attributes (10). Biological signals mediating cell-to-cell communication in prokaryotes and eukaryotes regulate diverse biological processes. In 2013, El Andaloussi et al., posited the nascent elucidation of pathophysiological roles for extracellular vesicles in the context of diseases including cancer, neurodegenerative disorders, and infectious diseases (5).

The Critical Role of Oocyte Maturation in Female Reproduction: Follicular Dynamics and The Emerging Role of Exosomes

Within higher organisms, the transmission of genetic information to subsequent generations is mediated by sperm and oocyte cells. Therefore, the maturation and functional competence of germ cells are of paramount importance for reproductive success and the propagation of genetic material (21). The initiation of successful female reproduction fundamentally relies on the extremely delicate developmental process of the oocyte cell. Oocyte maturation, a process preceding ovulation, entails the transition from prophase I to metaphase II of meiosis, a process initiated by the cessation of meiotic arrest. This process is essential for normal ovulation and fertilization, involving a shift in the balance between factors maintaining meiotic arrest and actively stimulating maturation. These events also represent a molecular signaling equilibrium (27). In 2023, Chen et al., stated that increasing evidence indicates exosomes' important functions in germ cell maturation (21).

The ovarian follicle represents the fundamental functional entity responsible for oocyte production and ensuing developmental processes (28), and it is also known to induce oocyte maturation and ovulation (29). Oocytes within mature ovarian follicles are surrounded by several cell layers. The innermost layer, directly apposed to the oolemma, is the zona pellucida. This extracellular matrix is ensheathed by the corona radiata granulosa cells and the cumulus granulosa cells. The antral cavity is filled with follicular fluid. The peripheral layer of the follicle is constituted by the theca cells, which are further organized into distinct outer and inner layers (30).

The Multifaceted Function of Exosomes in Ovarian Follicle Dynamics and Oocyte Maturation

The intricate process of intercellular communication within the follicle involves a diverse array of signaling molecules and maternal age is a critical influencing factor. Recent scientific literature indicates that the secretion of exosomes,

followed by their uptake by target cells, represents a mechanism for cell-cell interaction (31).

Granulosa cells control meiosis in ovarian follicles before ovulation (29) .

Exosomes facilitate communication between these cells and the oocyte (as well as theca cells), regulating oocyte metabolism and maturation, which represents a key step in this research. The transfer of exosomes between granulosa cells and the oocyte is shown in Figure-2.

In 2022, Kim et al., also supported this in their research that interaction between the ovarian follicle and cumulus cells is important for folliculogenesis, oocyte production, and fertilization capacity, and consequently, embryo development (32). Cellular interactions between oocytes and the cumulus corona complex are provided through gap junctions, which are intercellular channels (33). Intercellular channels contain connexins, including CX37 and CX43, which are known to play an active function in meiosis and oocyte maturation (34). In this process, the exosome-mediated expansion of the cumulus-oocyte-corona complex is integral to achieving successful oocyte maturation (35).

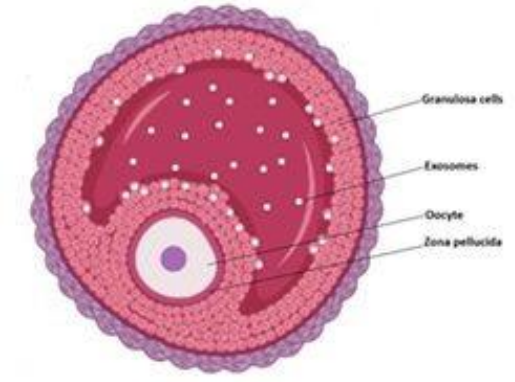

Figure-2. Diagrammatic illustration of exosome transfer between granulosa cells and oocyte (Created by using biorender.com and windows paint).

In their 2024 study using pigs, Ren et al., described the release of exosomes by granulosa cells. These exosomes from granulosa cells were taken up by cumulus-oocyte complexes through the follicular fluid. This finding corroborates the outcome of Wei et al.'s 2022 research, indicating that exosomes secreted by granulosa cells significantly enhance the rates of cumulus cell expansion and oocyte maturation. Correspondingly, the levels of harmful reactive oxygen species inside the cells decreased and the mitochondria performed better. These beneficial outcomes were due to increased cumulus cell expansion and the enhanced activity of genes involved in protecting the oocyte from stress (36,37).

Follicular exosomes are crucial for the ongoing process of meiosis in oocytes, the normal function of the ovaries, and preparing the fallopian tubes for fertilization (23). These extracellular vesicles function as carriers for bioactive molecules with the potential for oocyte targeting, likely to play a significant function in shaping the oocyte's RNA content and decisively influencing oocyte formation and early embryo development (38). Although the precise ways exosomal proteins (as cargo) directly control gene activity in the developing oocyte remain under investigation, their role in follicle-oocyte communication is confirmed (39).

The presence of exosomes in follicular fluids implies their influence on the follicular environment. Thus, further analysis of exosomal content is needed to investigate the hypothesis that they regulate oocyte maturation in this fluid and to improve our understanding of the maturation process.

In 2020, Shomali et al., demonstrated that exosomal miRNAs and mRNAs exert their effects on oocyte maturation via the MAPK, TGF-β, Wnt, and ubiquitin-mediated signaling cascade (40). In 2012, da Silveira et al., confirmed that some proteins secreted from granulosa cells support oocyte maturation, for instance Transforming Growth Factor-beta (TGF-β) family members are effective in this process (41).

Follicular fluid contains proteins, hormones, and exosomes in their cell secretions (40). Extracellular vesicles present within follicular fluid are characterized by a high content of ribosomes and RNA-binding proteins, these proteins exerting regulatory control over RNA gene expression and degradation processes. They can transfer ribosomal contents to the oocyte. The limited amount of mRNA in the follicular fluid suggests that these exosomes are released from other tissues and reach the follicular cells through circulation (42). Consistent with previous research, these results imply that the characteristics of exosomes can provide valuable insights into oocyte quality and its potential for successful development (43).

In 2015, Boots et al., stated in their study that cytokines released by granulosa cells were

defined as the principal component of the follicular environment (44). In 2021, Liu et al., reported that changes in the follicular environment affect the function of granulosa cells during oocyte maturation and lead to a slowdown in cell proliferation, eventually proving detrimental to oocyte growth and development (45).

Exosomes from follicular fluid, endometrium, embryo, and trophoblast cells can influence female infertility, implantation, and early pregnancy. Syncytiotrophoblast-derived exosomes exert a key function in later pregnancy stages (46). Bovine studies indicated that extracellular vesicles derived from the follicular fluid of 3-6 mm follicles promote oocyte maturation. Proteomic analysis of granulosa cells demonstrated a 41% concordance in protein content with the cargo proteins present in this follicular fluid (42). This suggests that exosomes have a therapeutic effect on restored cell activity. In contrast, in 2017, Matsuno et al., found that exosome-like vesicles isolated from porcine follicular fluids did not increase cumulus expansion (47). In addition, it has been shown that apoptosis seen in granulosa cells causes disruption of follicular development (48). In 2012, Collado-Fernandez et al., also demonstrated that extracellular vesicles mediate the exchange of biomolecules (including cytokines, enzymes, growth factors, and cell signaling proteins) between granulosa cells and the oocyte, modulating granulosa cell proliferation, steroid production, FSH response, and oocyte maturation (49).

As different approaches studies stated that the positive effects of extracellular vesicles depend on different follicle sizes (50,51). Consistent with this, in 2017, Hung et al., demonstrated in bovine models that exosomes originating from small follicles exhibited a greater capacity to enhance mural granulosa cell proliferation and growth than those originating from large follicles. The activity of extracellular vesicles within small antral follicles was shown to be associated with physiological conditions. However, no difference was observed in granulosa cell proliferative response to extracellular vesicles between different follicle sizes (51).

In 2002, Brännströ and Enskog, reported that a certain level of inflammatory stress is necessary for normal follicular development and ovulation, and this stress mediates to the growth and development of oocytes (52). This evidence suggests that granulosa cells, when exposed to oxidative stress, can release exosomes within the extracellular environment as a protective mechanism against potential apoptosis (53).

The Importance of Exosomes in Reproductive Medicine

A meticulous investigation into the function of exosomes within the reproductive system holds significant promise for bridging existing knowledge gaps. Extracellular vesicles facilitate intercellular communication and induce targeted cellular modifications via biomolecule transfer, positioning them as a significant tool in reproductive medicine.

Importantly, their role in oocyte and sperm maturation is essential for healthy embryo development. This function offers a new perspective for investigating previously unresolved questions in reproductive biology. Consequently, focused research on exosomes in reproductive medicine, particularly within the context of infertility, and the exploration of their therapeutic potential represent a compelling avenue for advancing *in vitro* fertilization (IVF) treatments.

In 2023, Zhou and Liu described that exosomes not only facilitate the elucidation of the molecular pathways participating in disease development but can also be harnessed as therapeutic agents in female infertility through activation by granulosa cells (53).

Therapeutic Applications of Exosomes in Reproductive Medicine

An expanding knowledge base regarding exosome function positions them as promising therapeutic modalities in reproductive medicine. Considering exosomes as novel therapeutic agents necessitates a detailed understanding of their content and target cell uptake, which will enable extensive clinical studies in cellular therapy. Moreover, considering exosomes as a cutting-edge therapeutic application in the absence of definitive retrospective data highlights the potentially growing importance of these extracellular vesicles. This suggests their increased recognition and application in future clinical settings. Within the *in vitro* fertilization (IVF) treatment paradigm, the implementation of natural autologous exosomes of patients as a contemporary strategy to procure superior quality and healthier embryos is a viable approach. Consequently, this could serve as a potential application for innovative and non-traditional

clinical research focused on improving the count and competence of oocytes.

The corroboration of positive outcomes in experimental investigations, particularly involving patients with diminished ovarian reserve (DOR), holds the potential to enhance the efficacy of infertility treatments by stimulating oocyte production. However, challenges in achieving the desired efficacy in specific target cells and their limited potency impede their widespread adoption in routine cellular therapies.

Chen et al., in 2021, emphasized that exosomes transmit the messages they contain as cargo molecules to the target cell through different methods. These methods can be listed as cell-to-cell and receptor associated endocytosis, phagocytosis, or micropinocytosis pathways, including various mechanisms such as surface receptor interaction and membrane fusion (10). A comprehensive understanding of exosome pathways prior to clinical translation directly impacts the efficacy of the intended results.

The increasing understanding of endogenous exosome functions reveals their biological properties as a continuing source of inspiration for scientific and clinical research. Their intrinsic properties, notably low immunogenicity and biological barrier permeability, exosomes offer functional cargo-carrying capabilities for therapeutic applications (54). In 2015, Haney et al., indicated in the same direction that the exosome-mediated delivery system includes large biomolecules for instance peptides, enzymes, antigens, cytoskeletal proteins, and transmembrane proteins (55).

Exosomes, when considered as carrier vehicles, have shown considerable promise in clinical therapeutics. Future advancements in exosome-based therapies require improved targeting of specific cells and tissues, along with prolonged systemic circulation (10).

Functionalizing exosomes with various active substances permits the attribution of specific biological functions. Competent encapsulation strategies are essential for the targeting of therapeutic cargo and the integration of targeting moieties within exosomes. Therefore, different methods that have been developed were shown in (Table-4).

Table-4. Classification of exosome loading methods.

Loading Methods
Biological Methods Simple incubation or complex viral transduction can be employed to load active molecules, such as therapeutic or targeting agents, with the choice of method dependent on the biological properties of the membrane and the vector (55,56).
Physical Methods Typical loading techniques include freeze-thaw cycles, sonication, electroporation, and extrusion (57).
Chemical Methods Methods e.g. saponin-assisted permeability and transfection facilitate the encapsulation of molecules through membrane permeability and electrostatic interaction. Studies indicate that chemical loading is often milder and more effective than physical approaches (58).

Current research primarily investigates the biodistribution and trafficking of exosomes in murine models. The optimization of cargo loading techniques and the advancement of bioengineering methodologies for membrane modification, aimed at prolonging circulation time and enhancing targeting capabilities, are crucial for the development of effective exosome-based delivery systems (10).

The Importance of Exosomes and Other Treatment Approaches in Premature Ovarian Insufficiency

Premature ovarian insufficiency (POI) is a typical endocrine disorder in women under 40, characterized by menstrual irregularities, infertility, anovulation, and hormonal imbalances (59). Factors causing POI include genetic mutations, impaired immune system, infections, and environmental factors such as chemotherapy. The major treatment measures for POI are hormone replacement therapy, immune regulation, ovarian transplantation and stem cell therapy (60).

Cho et al., in 2021, indicated that stem cell-derived extracellular vesicles are capable of promoting follicle development and improving ovarian function in a rat model of early ovarian insufficiency (60). Supporting this study in 2021 Zhang et al., conducted on rats with a stimulated premature ovarian insufficiency model, it was observed that concentrated exosomes derived from stromal cells obtained from menstrual blood improved ovarian activity. These exosomes

exhibit a positive impact, increasing follicle number, promoting folliculogenesis, improving ovarian morphology, regulating the estrous cycle and hormone profile, and enhancing live birth rates via the remodeling of the ovarian interstitial matrix (61).

In 2020 Seok et al., showed their study on rats that exosomes derived from placenta-derived mesenchymal stem cells (PD-MSCs) activate folliculogenesis and restore ovarian function after ovariectomy (62). In 2022 Qu et al., observed that human umbilical cord-derived exosomes mesenchymal stem cells (UCMSCs) increased the concentration of free amino acids, promoted angiogenesis and improved ovarian function in a rat model of POI (63).

In 2019 Huang et al., using human fetal mesenchymal stem cell (fMSCs) derived exosomes, conclude that these exosomes prevent follicle loss by reducing oxidative damage, inhibiting apoptosis, and restoring sex hormone levels (64). In the same year, Sun et al. demonstrated two key effects of bone marrow stem cell-derived exosomes in rats following the induction of premature ovarian failure. First, they restore endocrine functions, including the estrous cycle and sex hormone levels. Second, they augment primordial and antral follicle numbers. Functionally, these exosomes reduce the expression of apoptosis-related proteins and suppress granulosa cell apoptosis (65).

The Importance of Exosomes in Patients with Diminished Ovarian Reserve

Ovarian reserve, a complex measure of reproductive potential, is affected by age, environment, initial follicle count, diseases, drugs, and unknown factors. A characteristic of diminished ovarian reserve (DOR) in women is a lower follicle/oocyte number in comparison to women of the same age exhibiting normal ovarian function (66).

Through the action of their constituent miRNAs, exosomes target signaling cascades participating in follicular maturation and ovulation, consequently impacting granulosa cell proliferation, oocyte maturation, fertilization, as well as embryo development in the female genital tract (67). In 2023, Shen et al. reported that the exosomal miRNA expression within the follicular fluid environment of patients with human DOR significantly influences the pathological progression of this condition (68).

The regulation of mitochondrial functions by exosomes carrying mitochondrial RNA and its effect on patients with Diminished Ovarian Reserve is important, especially in oocyte development and fertility research. In 2024, Liu et al., indicates that the miRNA and mtRNA content of exosomes modulates mitochondrial function and energy metabolism in individuals with diminished ovarian reserve. Disruptions in this regulatory process can impair oocyte quality (69).

The Importance of Exosomes in Patients with Polycystic Ovary Syndrome

Investigations are also underway regarding the therapeutic use of EV in patients with polycystic ovary syndrome (PCOS). PCOS, disorder of the endocrine system, identified by complex metabolic dysfunctions, manifests clinically with features including menstrual irregularities, infertility, hirsutism and acne. Furthermore, endocrine dysregulation, hyperandrogenism, ovulatory dysfunction, polycystic ovarian morphology, obesity, and insulin resistance represent key diagnostic criteria observed in individuals with PCOS (70).

Follicle-stimulating hormone (FSH) is the primary stimulant of granulosa cells, and thecal cells are the principal site of estrogen synthesis. However, in endocrine disorders such as PCOS, decreased estrogen levels point to compromised granulosa cell activity. In 2016, Dewailly et al., emphasized that abnormally secreted hormones from granulosa cells are closely related to exosomes in PCOS patients and 16 differently expressed circular RNAs have been identified in follicular fluid exosomes of PCOS cases (71).

In 2023, Yu et al., demonstrated that exosomes within the follicular fluid of patients with PCOS are crucial for the regulation of oocyte maturation and the follicular microenvironment. Exosomal RNAs such as long non-coding RNA (lncRNA) and miRNA within the follicular fluids of PCOS patients have functions such as regulating intercellular communication and interaction. These RNAs are effective in lipid metabolism and involved in steroidogenesis and insulin resistance. Additionally, the alterations revealed through exosomal RNA profiling can contribute to potential diagnosis by elucidating the pathophysiology of PCOS (72).

The 2019 findings by Zhao et al. indicate the therapeutic prospect of EV in PCOS patients. Human adipose tissue mesenchymal stem cell-derived small extracellular vesicles (ADSC-sEVs)

suppress apoptosis and facilitate cumulus cell proliferation in this population. Moreover, ADSC-sEVs enhance the polycystic ovarian phenotype through the modulation of metabolic dysfunction and increasing reproductive function in a rat model of PCOS (73). In 2018, Kalhori et al. reported that the ameliorative effects of murine bone marrow-derived stem cells in PCOS were associated with a modest decrease in primordial and antral follicles, along with an increase in ovarian volume, cortex thickness, oocyte volume, and zona pellucida thickness (74).

Summary: Exosomes and Other Treatment Approaches in POI, DOR, and PCOS

Premature Ovarian Insufficiency (POI)

- **Characteristics:** Primary or secondary amenorrhoea accompanied by elevated circulating FSH (75).
- **Etiology**: Genetic mutations, immune dysfunction, infections, environmental factors (chemotherapy) (59) and medical interventions such as ovarian surgery or cytotoxic cancer therapy, metabolic and lysosomal storage diseases, chromosomal anomalies, and autoimmune diseases (75).
- **Role of Exosomes**:
  - EVs originating from stem cells facilitate folliculogenesis and ovarian role (60).
  - Menstrual blood stromal cell-derived exosomes improve follicle number, estrous cycle, and hormone profile (61).
  - Placenta-derived MSC exosomes restore ovarian function post-ovariectomy (62).
  - Umbilical cord MSC exosomes enhance amino acid levels, promote angiogenesis, and restore ovarian function (63).
  - Fetal MSC exosomes prevent follicle loss, reduce oxidative stress, and regulate hormone levels (64).
  - Bone marrow stem cell exosomes restore endocrine function and follicle numbers (65).

Diminished Ovarian Reserve (DOR)

- **Characteristics:** Decreased number and competence of follicles compared to age-matched women (66,76) and lower quality oocytes (76).
- **Etiology:** DOR originates from both endogenous and exogenous factors. Endogenous factors encompass modifications in follicular fluid constitution (proteins, fatty acids, amino acids, reduced choline/phosphocholine and lactate, elevated lysophosphatidylcholine, glucose, and HDL), alterations in vaginal microbiota, and autoimmune conditions (77).

- **Role of Exosomes**:
  - Exosomal miRNAs regulate follicular maturation, granulosa cell proliferation, oocyte maturation (67).
  - Altered exosomal miRNA profiles influence DOR pathology (68).
  - Exosomal miRNA and mtRNA regulate mitochondrial function and energy metabolism in oocytes (69).

Polycystic Ovary Syndrome (PCOS)

- **Characteristics**: Menstrual irregularity, infertility, hirsutism, acne, hyperandrogenism, ovulatory dysfunction, obesity, insulin resistance (70).
- **Etiology:** Endocrine dysregulation, hyperandrogenism, impaired ovulation, PCO morphology, obesity, and insulin resistance (70).
- **Role of Exosomes**:
  - Granulosa cell-secreted hormones and follicular fluid exosomal RNAs (miRNA, lncRNA) are altered (71).

- Exosomal RNAs regulate intercellular communication, lipid metabolism, steroidogenesis, insulin resistance (72).
- ADSC-sEVs suppress apoptosis, facilitate cumulus cell proliferation, alleviate metabolic dysfunction in PCOS models (73).
- Bone marrow-derived MSCs decrease follicle abnormalities and enhance ovarian structural features (74).

Limitations and Future Perspectives

EVs are key players in intercellular signaling via bioactive molecule transmission and demonstrate therapeutic potential due to their biocompatibility and other advantages. They have potential in treating female reproductive disorders (70).

Exosomes are of significant interest as native carriers due to enhancements in their biocompatibility, capacity for profound tissue permeation, versatile cargo loading capability, and resilience to surface alteration. Exosome engineering, which involves incorporating cell-specific targeting ligands or synthetic polymeric materials, enables precise targeting and extends circulation while preserving their inherent characteristics (10). Exosomes and their cargo are a new research focus with potential as markers for infertility evaluation and treatment, owing to their widespread presence *in vivo* and accessibility, indicating a bright future in reproduction (78). To improve exosomal isolation and identification, and to segregate nascent exosomes associated with reproductive disorders, next-generation sequencing and nanoparticle-tracking analysis are at the vanguard of biomedical technology (79). Nevertheless, metric research on exosomes is essential for further investigation, particularly regarding their application in the early diagnosis and therapy of disorders (80). Current exosome research is still in its nascent stages, with advanced exosome manufacturing and storage technologies remaining challenging (81).

CONCLUSION

The clinical translation of exosomes as a therapeutic modality necessitates a comprehensive elucidation of their potential adverse effects. The use of autologous exosomes may mitigate certain risks. Future research should prioritize studies in large animal models, followed by human clinical trials to facilitate practical implementation. Expanding knowledge of exosome biology is expected to yield further insights into oocyte maturation, potentially enabling the development of novel therapeutic modalities for fertility impairments such as diminished ovarian reserve and premature ovarian insufficiency.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Abbreviations

ADSC-sEV	Adipose mesenchymal stem cell-derived small extracellular vesicle
CX37	Connexin 37
CX43	Connexin 43
DNA	Deoxyribonucleic acid
DOR	Diminished ovarian reserve
ESCRT	Endosomal sorting complexes required for transport called exosome formation and release
EV	Extraselullar vesicle
fMSCs	Fetal mesenchymal stem cells
FSH	Follicle-Stimulating hormone
IVF	*In vitro* fertilization
lncRNA	Long non-coding RNA
MAPK	Mitogen-activated protein kinase

mRNA	Messenger RNA
miRNA	Micro RNA
mtRNA	Mitochondrial RNA
MV	Microvesicle
MVBs	Multivesicular bodies
PCOS	Polycystic Ovarian Syndrome
PD-MSCs	Placenta-derived mesenchymal stem cells
POI	Premature ovarian insufficiency
TGF-β	Transforming Growth Factor-β
UCMSCs	Umbilical cord mesenchymal stem cells
Wnt	Wingless-related integration site

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