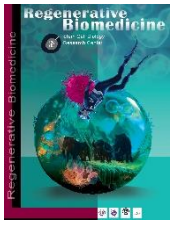

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Review Article

Nanoengineered Exosome Platforms for Targeted Ovarian Regeneration in Premature Ovarian Insufficiency

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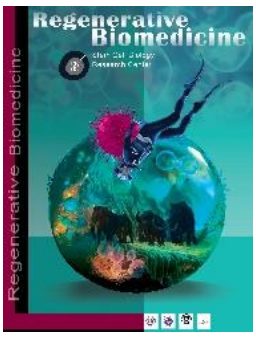
Abstract

Premature Ovarian Insufficiency (POI), is a clinically significant reproductive endocrine disorder defined by the cessation of ovarian function prior to age 40, with a global prevalence of approximately 1–2% among women of reproductive age. The condition manifests as a constellation of reproductive and systemic sequelae, including infertility, hypoestrogenism, accelerated bone loss, heightened cardiovascular risk, and considerable psychosocial burden. While current standard of care predominantly centers on hormone replacement therapy, which merely compensates for hormonal deficiency without addressing the underlying pathological process of follicular depletion, nanoengineered exosome platforms have emerged as a paradigm-shifting therapeutic opportunity for restoring functional ovarian capacity. This comprehensive review synthesizes advances published between 2022 and 2026 in the evolving landscape of nanoengineered exosome-based interventions for POI. Exosomes represent superior alternatives to intact mesenchymal stem cells due to their inherent biocompatibility, low immunogenicity, ability to traverse biological barriers, and greater potential for standardization. Recent developments in advanced cargo loading technologies (CRISPR-Cas9-mediated genetic engineering of parent MSCs, and sonication-mediated encapsulation of small-molecule therapeutics), surface engineering strategies (ovarian homing peptides, AMHR2-targeting aptamers, and bio orthogonal click chemistry conjugation), and hybrid nanoplatforms (exosome-liposome hybrids, ROS-responsive polymer coatings, and injectable thermosensitive hydrogel composites) are critically evaluated. The evidence base has substantially advanced both mechanistic understanding and engineering capabilities, with CRISPR-guided cargo enrichment, aptamer-based ovarian targeting, and stimuli-responsive polymer-exosome hybrids representing standout developments. As these technologies mature, nanoengineered exosomes stand as one of the most promising vehicles for achieving genuine ovarian regeneration in POI transitioning from theoretical aspiration toward clinical reality.

Keywords: CRISPR-Cas9, Exosomes, Infertility, Premature Ovarian Insufficiency, Nano Platforms



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Introduction

Premature Ovarian Insufficiency (POI), previously termed premature ovarian failure, is a clinically significant reproductive endocrine disorder defined by the cessation of ovarian function prior to age 40 (1). Epidemiological data indicate a global prevalence of approximately 1–2% among women of reproductive age (2). The condition manifests as a constellation of reproductive and systemic sequelae, including infertility, hypoestrogenism, accelerated bone loss, heightened cardiovascular risk, and considerable psychosocial burden (3). The etiological landscape of POI is heterogeneous, encompassing chromosomal abnormalities, single-gene mutations, autoimmune pathology, iatrogenic insults from chemotherapy or radiotherapy, and a substantial proportion of idiopathic cases (4). The current standard of care for POI centers predominantly on hormone replacement therapy (HRT) to compensate for estrogen and progesterone deficiency, thereby mitigating symptomatic burden and protecting against downstream organ complications (5). However, HRT fundamentally fails to address the primary pathological process (follicular depletion and granulosa cell dysfunction) and is associated with long-term risks including venous thromboembolism (6). Oocyte donation represents the principal fertility treatment option, yet it precludes genetic motherhood and does not reverse the endocrine deficiency or associated systemic consequences (7). These limitations underscore an urgent, unmet clinical need for therapies that target the root pathophysiology of POI to restore functional ovarian capacity (8). Over the past decade, regenerative medicine approaches (most notably mesenchymal stem cells (MSC)-based therapies) have generated substantial scientific interest as candidate interventions for POI (9). MSCs, derived from bone marrow, adipose tissue, umbilical cord, and other sources, possess potent immunomodulatory, anti-apoptotic, and pro-angiogenic properties that can favorably remodel the damaged ovarian microenvironment (10). Preclinical studies have consistently demonstrated improvements in follicular reserve, hormone secretion, and even restoration of spontaneous fertility following MSC transplantation in chemotherapy-induced or genetic POI models (11). Nonetheless, direct MSC transplantation carries risks of immune rejection, tumor formation, embolic complications, and uncertain long-term engraftment, collectively limiting their clinical utility (12). A paradigm shift in the understanding of MSC therapeutic mechanisms has revealed that the paracrine secretome (particularly exosomes) accounts for a substantial proportion of their regenerative effects (13). Exosomes are membrane-bound extracellular vesicles (30–150 nm diameter) produced via the endosomal pathway and released by virtually all mammalian cell types (14). They function as sophisticated intercellular messengers, transferring a complex molecular cargo of proteins, lipids, mRNAs, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) to recipient cells, thereby modulating diverse biological processes (15). Unlike intact MSCs, exosomes are non-replicative, exhibit low immunogenicity, readily traverse biological barriers, and can be stored and standardizedmore tractably, attributes that

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render them highly attractive as clinical therapeutics (16). Despite these intrinsic advantages, native MSC-derived exosomes exhibit several limitations that constrain their clinical translation (17). Their therapeutic payload may be insufficient or poorly defined, ovarian targeting is inherently non-specific, and production at clinically relevant scale under regulatory-compliant conditions remains technically demanding (18). Nanoengineering has emerged as the pivotal discipline to overcome these constraints (19). Through precise manipulation of exosome cargo composition, membrane surface properties, and structural architecture, nanoengineering enables the creation of next-generation therapeutic vesicles with programmable targeting, enhanced potency, and optimized pharmacokinetics (20). This review provides a comprehensive synthesis of the evolving landscape of nanoengineered exosome platforms for POI, integrating advances published between 2022 and 2026 (21). We systematically examine the pathophysiology of POI, the biology of exosomes as therapeutic agents, recent developments in cargo engineering and surface functionalization, strategies for targeted ovarian delivery, and the translational challenges that must be surmounted to realize the clinical potential of these platforms. The overall therapeutic concept and mechanistic pathways of nanengineered exosome-based interventions for premature ovarian insufficiency are summarized in the graphical abstract.

Pathophysiology of Premature Ovarian Insufficiency

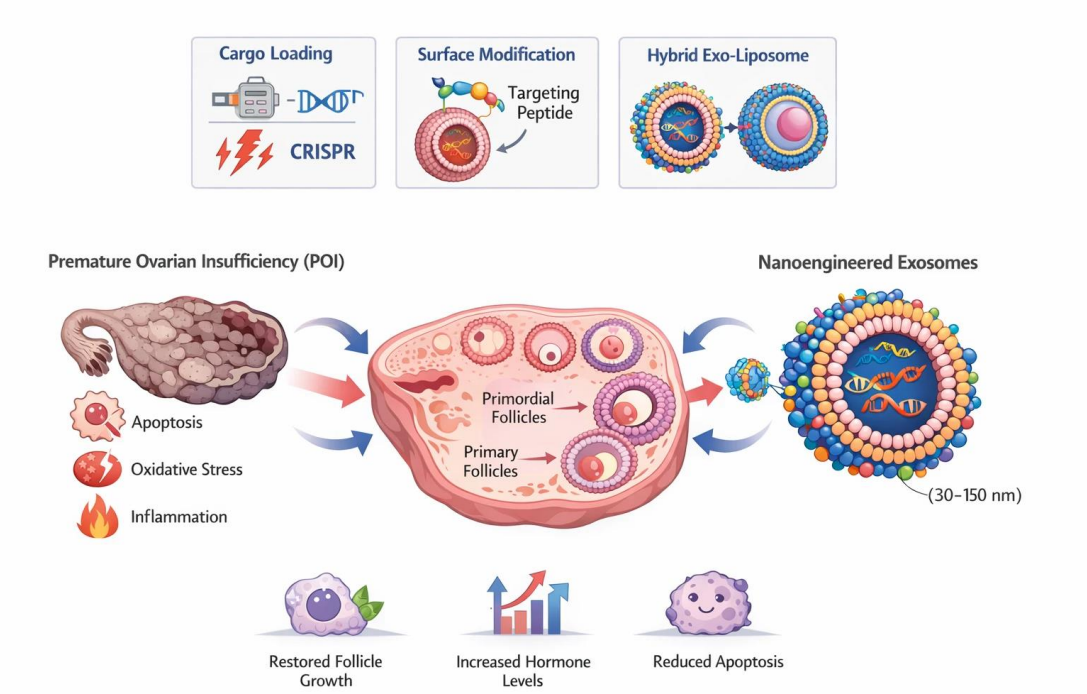
The pathophysiology of POI is multidimensional, involving interacting mechanisms that collectively accelerate the loss of functional ovarian tissue and disrupt the hormonal milieu required for reproductive and systemic health (22).

Accelerated Follicular Depletion and Primordial Follicle Exhaustion

The human ovarian reserve, established during fetal development, consists of a finite cohort of primordial follicles that are progressively recruited and lost throughout reproductive life. In POI, this physiological attrition is dramatically accelerated, either through heightened follicular atresia or diminished primordial follicle activation (4). Single-cell transcriptomic analyses published since 2022 have provided unprecedented resolution of the molecular events governing primordial follicle dormancy and activation (23). Studies have identified dysregulated PI3K/PTEN/AKT signaling as a central driver of inappropriate follicle awakening, leading to premature exhaustion of the reserve (6). Additionally, mutations in NOBOX, FIGLA, and GDF9 disrupt oocyte-somatic cell crosstalk critical for follicle maintenance (4). Epigenetic dysregulation, including aberrant DNA methylation and histone modification patterns in oocytes, has also been increasingly implicated in premature follicular loss (6).

Granulosa Cell Apoptosis and Dysfunctional Steroidogenesis

Granulosa cells (GCs) constitute the primary functional cell population supporting folliculogenesis and steroidogenesis. In POI, GCs are disproportionately vulnerable to apoptotic stimuli, including oxidative stress, growth factor deprivation, and inflammatory cytokines (21, 22). Mechanistic studies have elucidated that the intrinsic apoptotic



Graphical Abstract : Schematic illustration of nanengineered exosome-based therapeutic strategies for premature ovarian insufficiency (POI). Engineered exosomes, including hybrid exo-liposomes with cargo loading (e.g., CRISPR), surface modification, and targeting peptides, alleviate apoptosis, oxidative stress, and inflammation in ovarian tissue. This leads to restoration of primordial and primary follicle development, increased hormone levels, and improved ovarian function.

pathway, mediated Mechanistic studies have elucidated that the intrinsic apoptotic pathway, mediated through Bcl-2 family protein imbalance and mitochondrial cytochrome c release, predominates in GC loss (22). This apoptotic cascade severely impairs steroidogenesis, reducing estradiol (E2) and anti-Müllerian hormone (AMH) production, which are the principal hormonal biomarkers of ovarian reserve and function (8).

Oxidative Stress and Mitochondrial Dysfunction

The ovarian microenvironment is inherently susceptible to oxidative stress due to the high metabolic demands of folliculogenesis and steroidogenesis. In POI, an imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms leads to cumulative oxidative damage (12). Mitochondrial dysfunction is both a consequence and amplifier of this oxidative burden (10, 12). Recent investigations using mitochondrial transcriptomic profiling in POI-derived GCs have revealed impaired electron transport chain complex activity, decreased mitochondrial membrane potential, and elevated mitochondrial ROS generation (6). These findings establish mitochondrial health as a critical therapeutic target in POI, with exosome-delivered mitochondria-protective cargoes representing a promising intervention (12).

Immune Dysregulation and Chronic Ovarian Inflammation

Immune-mediated ovarian damage is increasingly recognized as a prevalent and

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underdiagnosed contributor to POI pathogenesis. Autoimmune POI (associated with anti-oocyte and anti-steroidogenic cell antibodies) accounts for a significant minority of cases, while subclinical immune activation appears relevant across broader POI subtypes (7). Elevated levels of pro-inflammatory cytokines, including TNF-α, IL-6, IL-1β, and interferon-γ, have been documented in the follicular fluid and systemic circulation of POI patients, correlating with reduced follicle counts and deteriorating AMH levels (6). Emerging data from single-cell immune profiling studies have demonstrated aberrant macrophage polarization toward inflammatory (M1) phenotypes within POI ovaries, driving a cycle of tissue destruction that perpetuates follicular loss (23). Restoration of immune homeostasis through immunomodulatory exosome cargo therefore represents a critical therapeutic objective (16).

DNA Damage Accumulation and Defective Repair

Oocytes are among the most long-lived cells in the human body and are particularly susceptible to cumulative DNA damage from endogenous and exogenous sources. Double-strand breaks (DSBs), single-strand breaks, and base modifications accumulate with advancing age and following gonadotoxic exposures (4). Deficiencies in homologous recombination repair (particularly loss-of-function variants in BRCA2, BRIP1, and MCM8/9) have been recently identified as significant genetic risk factors for POI, underscoring the relevance of impaired DNA damage response pathways (6). Persistent DNA damage activates p53-p21 and ATM-CHK2 checkpoints, inducing GC senescence and apoptosis, further depleting the functional ovarian reserve (22).

Exosomes as Natural Nanocarriers: Biology and Therapeutic Rationale

Biogenesis and Molecular Composition

Exosomes are generated through a tightly regulated endosomal pathway. Early endosomes mature into late endosomes (multivesicular bodies, MVBs) through sequential membrane invaginations that generate intraluminal vesicles (ILVs) (11, 13). MVBs subsequently fuse with the plasma membrane, releasing ILVs as exosomes into the extracellular milieu (13). This process is orchestrated by the endosomal sorting complex required for transport (ESCRT) machinery as well as ESCRT-independent mechanisms involving ceramide, tetraspanins, and the lipid raft pathway (14). The molecular cargo of exosomes is selectively enriched and reflects the physiological state of the parent cell (13, 14). It encompasses a dynamic repertoire including tetraspanins (CD9, CD63, CD81), heat shock proteins (HSP70, HSP90), cytoskeletal proteins, growth factors, signaling kinases, lipids (cholesterol, sphingomyelin, phosphatidylserine), and a diverse nucleic acid payload (13). The nucleic acid cargo (comprising mRNAs, miRNAs, lncRNAs, circRNAs, and genomic DNA fragments) represents the primary effector mechanism through which exosomes modulate recipient cell gene expression and phenotype (11).

Advantages Over Synthetic Nanoparticle Systems

The past three years have witnessed intensified comparative analyses of exosomes

versus conventional synthetic delivery platforms, including lipid nanoparticles (LNPs), polymeric nanoparticles (PNPs), and dendrimers. Exosomes offer distinct biological advantages: their phospholipid bilayer is structurally homologous to cellular membranes, conferring inherent biocompatibility and facilitating efficient membrane fusion with target cells (17). Their surface proteome contains targeting moieties (integrins, selectins, cell adhesion molecules) that mediate tissue tropism (18). Furthermore, exosomes display substantially lower immunogenicity than synthetic vectors, an attribute of considerable importance for repeated dosing regimens envisioned in chronic conditions like POI (15).

Notwithstanding these advantages, native exosomes exhibit intrinsic limitations that motivate nanoengineering interventions. Endogenous exosome yields from conditioned cell culture media are typically low, their cargo composition is heterogeneous and may include undesirable molecular species, and their biodistribution following systemic administration is dominated by hepatic and splenic clearance via the reticuloendothelial system (RES), limiting ovarian accumulation (24). These limitations collectively define the engineering objectives that nanoengineered exosome platforms seek to address (18).

Therapeutic Effects of Native MSC-Derived Exosomes in POI

Bone Marrow MSC-Derived Exosomes (BMSC-Exos)

BMSC-derived exosomes have been the most extensively investigated in POI preclinical models. Recent studies demonstrated that BMSC-Exos administered to cyclophosphamide-induced POI mice significantly increased primordial and primary follicle counts, elevated serum AMH and E2 levels, and restored estrous cyclicity (5). Mechanistic dissection attributed these effects to exosomal miRNAs which suppressed pro-apoptotic genes in GCs, thereby enhancing mitochondrial function and reducing apoptosis (12).

Umbilical Cord MSC-Derived Exosomes (UC-MSC-Exos)

UC-MSCs represent an ethically accessible, neonatal-sourced cell population with potent immunomodulatory capacity. UC-MSC-derived exosomes have demonstrated therapeutic efficacy in multiple POI models (25). A 2022 study showed that UC-MSC-Exos improved ovarian function in POI mice by targeting the Wnt/β-catenin signaling pathway (5, 25). The therapeutic mechanism was linked to the abundant miR-29a cargo of UC-MSC-Exos, which inhibited pro-apoptotic genes and consequently activated GC survival pathways (25).

Adipose-Derived Stem Cell Exosomes (ADSC-Exos)

ADSCs offer a clinically practical, abundantly available source of therapeutic exosomes obtainable through minimally invasive lipoaspiration (10). ADSC-Exos have demonstrated multifaceted protective effects in POI models, encompassing anti-oxidative, anti-apoptotic, and pro-angiogenic activities (2, 10). In vivo, ADSC-Exos significantly improved ovarian microvascular density and follicular vascularity in POI models, suggesting that restoration of ovarian blood supply is a key mechanism of their regenerative action (10).

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Functional Ovarian Outcomes Across MSC-Exosome Types

Evidence synthesized from 2022 to 2025 across multiple preclinical studies involving MSC-Exos in POI models consistently documented meaningful improvements in: (i) serum AMH levels, (ii) restoration of estradiol secretory capacity, and (iii) total antral follicle counts (8). These functional endpoints align with clinically validated biomarkers used in human POI diagnosis and monitoring per international guidelines, strengthening the translational relevance of preclinical findings (2).

Exosomal RNA Cargo in Ovarian Regeneration

MicroRNAs as Primary Effector Molecules

MiRNAs represent the most extensively characterized class of exosomal effectors in ovarian regeneration (12). The miR-21 family occupies a central position in exosome-mediated GC cytoprotection, functioning through simultaneous suppression of multiple pro-apoptotic targets including PDCD4 and PTEN (17, 12). A 2023 multi-center in vitro study demonstrated that exosomes engineered to overexpress miR-21 reduced doxorubicin-induced GC apoptosis compared to unmodified exosomes, while preserving steroidogenic enzyme expression (18). The miR-17-92 cluster has been demonstrated to promote GC proliferation by targeting the PTEN-AKT axis and cell cycle inhibitors (p21, p27) (19). Single-molecule sequencing of exosomal miRNA from human BMSC cultures identified significant enrichment of this cluster under hypoxic preconditioning, with hypoxia-primed exosomes exhibiting superior efficacy in promoting follicle development (12). MiR-146a-5p has emerged as a critical regulator of ovarian immune homeostasis, targeting TRAF6 and IRAK1 in the NFκB signaling cascade to attenuate GC inflammatory responses (12). Additional miRNAs implicated in exosome-mediated ovarian regeneration include miR-let-7, miR-29b, and miR-200c (18).

Long Non-Coding RNAs (lncRNAs)

LncRNAs (defined as non-coding transcripts exceeding 200 nucleotides) have been identified as critical regulatory molecules within the exosomal cargo of MSCs (11). The lncRNA NEAT1, detected in BMSC-Exos, has been shown to act as a competing endogenous RNA (ceRNA) sequestering miR-155, thereby de-repressing anti-apoptotic BCL-2 expression in GCs under oxidative stress conditions (12). LncRNA LINC00114, transferred via exosomes from endometrial stromal cells to GCs, has been demonstrated to repress FOXO3a-driven follicular atresia, establishing a novel intercellular axis with direct therapeutic relevance to POI (5).

Circular RNAs (circRNAs)

CircRNAs (covalently closed RNA structures generated by back-splicing of pre-mRNA) have emerged as a functionally significant component of the exosomal RNA landscape (11). Their covalently closed architecture confers resistance to RNA exonucleases, enabling prolonged biological activity following exosomal delivery (18). CircRNA ciRS-7 (also termed CDR1as), identified in MSC-Exos, functions as a miR-7 sponge that indirectly upregulates IRS2/PI3K/AKT signaling in GCs, promoting their survival under genotoxic stress (12). The stability and functional versatility of exosomal circRNAs

position them as high-value candidates for engineered cargo enrichment strategies (19).

Nanoengineering Strategies for Enhanced Exosome Therapy

Advanced Cargo Loading Technologies

Efficient and reproducible cargo loading is a foundational prerequisite for therapeutic exosome manufacturing (18). Classical electroporation (application of brief high-voltage electrical pulses to transiently permeabilize the exosome membrane) remains widely utilized but has been associated with exosome aggregation, membrane disruption, and inconsistent loading efficiency (18). A 2023 methodological comparison found that microfluidic electroporation using an integrated chip-based platform achieved higher miRNA loading efficiency with preserved exosome integrity, compared to bulk electroporation (26). CRISPR-Cas9-mediated genetic engineering of parent MSCs represents the most sophisticated approach to cargo programming (26, 27). By genomic integration of synthetic miRNA expression cassettes under constitutive or inducible promoters, MSCs can be programmed to constitutively secrete exosomes enriched with therapeutic miRNA cocktails (27). A 2024 proof-of-concept study demonstrated that CRISPR-engineered BMSCs overexpressing therapeutic miRNAs produced exosomes with higher loading of each miRNA and significantly superior GC-protective efficacy in vitro compared to parental cell exosomes (26). Sonication-mediated passive loading and co-incubation methodologies have been optimized for small-molecule drug encapsulation within exosomes (18, 27). A 2024 study demonstrated efficient encapsulation of therapeutic agents into BMSC-Exos via saponin-assisted permeabilization, with resulting exosomes demonstrating superior mitochondrial protective effects in damaged GCs compared to free drug (18).

Surface Engineering for Targeted Ovarian Delivery

The engineering of exosome surface properties to confer ovarian tropism is a rapidly advancing domain. Peptide-based targeting moieties identified through phage display and in silico peptide-receptor docking have been conjugated to exosome surfaces via several bioconjugation chemistries (21). Aptamer-functionalized exosomes represent a rapidly evolving targeting strategy. DNA aptamers selected via systematic evolution of ligands by exponential enrichment (SELEX) against ovarian cell antigens have been repurposed and refined for targeting receptors on GCs (28). Click chemistry (specifically strain-promoted azide-alkyne cycloaddition (SPAAC) and tetrazine-trans-cyclooctene (Tz-TCO) ligation) has been established as a robust, bioorthogonal platform for site-specific exosome surface functionalization (21). These reactions proceed efficiently in aqueous conditions without toxic metal catalysts, preserving exosome viability (21, 28). A 2023 optimization study employed metabolic glycan labeling of MSCs with azide-modified mannosamine, generating exosomes with surface-displayed azide handles for subsequent SPAAC conjugation of targeting ligands with high conjugation efficiencies (21).

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Hybrid Nanoplatforms: Integrating Exosomes with Synthetic Nanomaterials

Hybrid nanoplatforms, created by integrating exosomes with synthetic nanomaterials, represent the most structurally sophisticated approach to exosome engineering and have attracted considerable research investment since 2022 (29). Exosome-liposome hybrids generated through microfluidic-assisted membrane extrusion combine the targeting specificity of the exosome proteome with the high drug encapsulation capacity of liposomal carriers (30). A 2023 in vitro study demonstrated that exosome-liposome hybrids co-loaded with therapeutic agents showed synergistic GC protective effects, reducing apoptosis compared to individual nanocarriers alone (29). Polymer-exosome hybrid systems have been developed to extend the circulatory half-life of therapeutic exosomes and enable stimuli-responsive cargo release (31). Coating exosomes with pH-sensitive poly(lactic-co-glycolic acid) (PLGA) shells confers protection against lysosomal degradation following cellular uptake, releasing cargo preferentially in the acidic endo-lysosomal compartment (30, 31). ROS-responsive hyaluronic acid (HA)-coated exosomes that selectively release their miRNA cargo in the oxidatively stressed ovarian microenvironment characteristic of POI have demonstrated enhanced therapeutic efficacy compared to uncoated exosomes (32).

Exosome-hydrogel composite systems represent a further evolution in local ovarian delivery (32, 33). Injectable thermosensitive hydrogels loaded with therapeutic exosomes have been developed for intraovarian administration, providing a depot-release platform that maintains high local exosome concentrations over extended periods (33).

Targeted Ovarian Delivery Strategies

Routes of Administration: Systemic versus Local

The choice of administration route critically determines the pharmacokinetics and therapeutic efficacy of exosome-based interventions. Intravenous (IV) administration offers clinical convenience and is amenable to repeated dosing, but is challenged by rapid RES clearance (hepatic Kupffer cells, splenic macrophages) and nonspecific biodistribution (17). Pharmacokinetic modeling studies have demonstrated that unmodified exosomes exhibit plasma half-lives of 30–120 minutes following IV injection in rodents, with ovarian accumulation accounting for less than 2% of the administered dose (14). Local delivery approaches substantially overcome these biodistribution limitations (8). Intraovarian injection, while technically demanding and requiring laparoscopic or ultrasound-guided access, achieves direct tissue-level concentrations several orders of magnitude higher than IV delivery (8, 14). Emerging minimally invasive alternatives, including transvaginal ultrasound-guided ovarian injection (analogous to established oocyte retrieval procedures) are being developed to translate intraovarian delivery into clinical practice with acceptable procedural risk profiles (8).

Biodistribution Optimization and Stealth Engineering

Extending exosome circulatory half-life to improve passive ovarian accumulation is

achievable through surface stealth engineering (21). Conjugation of polyethylene glycol (PEG) chains to the exosome surface (PEGylation) reduces complement activation and macrophage recognition, prolonging plasma residence time (5, 21). However, excessive PEGylation can impair cellular uptake; optimization of PEG chain length and surface density is therefore critical (21). CD47 ('don't eat me signal') overexpression on the exosome surface, achieved through parent cell genetic engineering, has been demonstrated as an alternative stealth strategy, reducing phagocytic clearance and improving tissue accumulation (18).

Active Targeting Mechanisms

Active targeting through surface-displayed ovarian homing moieties represents the most precise approach to increasing ovarian exosome accumulation (21). Beyond general targeting peptides, research has identified targeting moieties for ovarian endothelial surface markers, including CD105 (endoglin) and VEGFR2, whose expression is upregulated in the developing ovarian vasculature (34). Dual-targeting strategies, equipping exosomes with both vascular endothelial targeting peptides (for extravasation) and GC-targeting aptamers (for cellular uptake), have been developed to create hierarchical targeting systems that navigate the multiple biological barriers between the systemic circulation and individual GCs (28).

Stimuli-Responsive Release in the Ovarian Microenvironment

The ovarian microenvironment in POI is characterized by elevated ROS, mild acidity in atretic follicles, and upregulated matrix metalloproteinase (MMP) activity (31). These biochemical signals have been exploited as endogenous triggers for context-specific cargo release from engineered exosome systems (23, 31). MMP-2/9-cleavable peptide linkers incorporated between targeting ligands and exosome surfaces enable selective release of surface-conjugated therapeutic molecules specifically within follicular fluid of degenerating follicles (30-31). Similarly, ROS-responsive boronate ester linkages incorporated into hybrid exosome-polymer coatings have been demonstrated to enable hydrogen peroxide-triggered cargo release specifically in oxidatively stressed ovarian tissue, with minimal premature release in healthy tissues (31).

Translational and Manufacturing Challenges

GMP-Compliant Production Systems

The transition of exosome therapeutics from laboratory-scale research to clinical-grade production necessitates compliance with Good Manufacturing Practice (GMP) regulations enforced by the FDA, EMA, and equivalent international agencies (15, 35). GMP-compliant exosome manufacturing requires standardized, traceable master cell banks of parent MSCs, closed-system bioreactor-based cell culture platforms, and validated downstream processing protocols (35). Current challenges include the absence of universally accepted reference standards for exosome characterization, reliance on ultracentrifugation-based isolation that introduces batch-to-batch variability, and insufficient analytical tools for real-time potency monitoring (15). Tangential flow filtration (TFF) and size exclusion chromatography (SEC) have emerged as preferred GMP-compatible alternatives to

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ultracentrifugation, offering superior scalability and reproducibility (24). A 2024 GMP process development study achieved high purity and particle yield using a combined TFF/SEC workflow from UC-MSC hollow fiber bioreactor cultures, representing a significant milestone toward clinical-scale production (24). The International Society for Extracellular Vesicles (ISEV) published updated minimum quality criteria (MQC) in 2023 specifically addressing therapeutic EV manufacturing, providing a regulatory-aligned quality framework (36).

Scalability and Cost-Effectiveness

Economic viability is a critical but often underappreciated dimension of exosome therapeutic development (35). Cost modeling analyses published in 2024 estimated that current clinical-scale exosome manufacturing costs range from USD 2,000–8,000 per patient dose, largely driven by bioreactor consumables, downstream processing reagents, and quality control analytics (35, 37). Strategies to reduce costs include: (i) transitioning from 2D culture flasks to 3D dynamic bioreactor systems (hollow fiber, stirred tank) that increase volumetric exosome yields 20-100-fold, (ii) developing continuous manufacturing platforms that integrate cell culture, harvest, and purification in a unified automated process, and (iii) employing exosome-mimetic nanovesicles (nanoparticles generated by serial cell membrane extrusion) which can be produced at higher yields than biological exosomes while preserving key surface properties (24).

Long-Term Storage Stability

Maintaining exosome structural integrity and biological potency during storage is essential for practical clinical deployment (38). Standard storage at -80°C with phosphate-buffered saline preserves exosome function for up to 6 months but is compromised by repeated freeze-thaw cycles that induce membrane fusion and aggregation (39). Lyophilization (freeze-drying) with appropriate cryoprotectants (sucrose, trehalose, mannitol) has been demonstrated to extend shelf-life to 12–18 months at 4°C without significant loss of biological activity (40). A 2024 optimization study employing microfluidic spray freeze-drying with trehalose achieved high structural preservation and miRNA cargo retention after lyophilization, representing the current state-of-the-art for therapeutic exosome formulation stability (41).

Regulatory Pathways and Ethical Considerations

The regulatory classification of therapeutic exosomes remains an evolving and internationally inconsistent landscape (42). In the United States, the FDA currently classifies most therapeutic exosomes as biological products subject to BLA (Biologics License Application) review, while acknowledging that engineered exosomes incorporating gene therapy cargoes may additionally require IND (Investigational New Drug) filing under CBER oversight (43). The EMA has similarly categorized MSC-derived exosomes as Advanced Therapy Medicinal Products (ATMPs), imposing stringent European regulatory requirements (44). The ISEV Regulatory Affairs taskforce, in a 2024 position paper, advocated for a harmonized international regulatory

framework specifically tailored to extracellular vesicle therapeutics, emphasizing the need for potency assay standardization, donor screening protocols, and risk-stratified clinical trial design (45). Ethical dimensions (including informed consent requirements for MSC donors, equitable access considerations given anticipated manufacturing costs, and the management of incidentally identified genetic variants in donor cell characterization) have been addressed in recent bioethics frameworks specifically developed for exosome therapeutics in reproductive medicine (46).

Future Perspectives

Personalized Exosome Medicine for POI

The etiological heterogeneity of POI strongly advocates for personalized therapeutic approaches that account for individual genetic, immunological, and ovarian reserve profiles (6). The emerging paradigm of 'theranostic exosomes' (vesicles simultaneously functionalized for diagnostic biomarker detection and therapeutic cargo delivery) could enable real-time monitoring of ovarian response to exosome therapy using surface-bound biosensors (17). Autologous exosome therapy, in which exosomes derived from the patient's own reprogrammed somatic cells or induced pluripotent stem cell (iPSC)-derived MSCs are returned to the patient, offers maximal immunocompatibility and the potential for patient-specific cargo optimization (11).

Artificial Intelligence in Exosome Engineering

Artificial intelligence (AI) and machine learning (ML) are increasingly being integrated into exosome research workflows, offering transformative capabilities for therapeutic optimization. Deep learning models trained on multi-omic datasets from MSC-Exo studies have been developed to predict exosome cargo composition based on parent cell culture conditions, enabling computational optimization of production parameters (47). Generative AI approaches, including variational autoencoders (VAEs) applied to miRNA sequence-function data, have been employed to design novel synthetic miRNA sequences with predicted superior anti-apoptotic efficacy in GCs, transcending the limitations of naturally occurring miRNA sequences (48). Reinforcement learning algorithms have also been applied to optimize electroporation parameters for therapeutic nucleic acid loading into exosomes, achieving loading efficiencies superior to manually optimized protocols (49).

Combination and Multimodal Therapeutic Strategies

The multifactorial pathophysiology of POI is unlikely to be fully addressed by any single therapeutic modality (8). Combinatorial approaches integrating nanoengineered exosomes with complementary therapeutic platforms are increasingly being explored (50). The integration of exosome therapy with platelet-rich plasma (PRP) (which delivers autologous growth factors including PDGF, TGF-β, and VEGF) has demonstrated synergistic follicular preservation in clinical pilot studies (51). Combinations of exosome therapy with low-intensity laser therapy (LILT), which promotes mitochondrial biogenesis and reduces oxidative stress in ovarian tissue, are being investigated as non-pharmacological synergistic approaches (52).

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Clinical Trial Design and Regulatory Roadmap

Phase I clinical trials investigating MSC-derived exosome safety in POI patients were registered in international trial registries between 2023 and 2025, primarily evaluating intraovarian injection of BMSC-Exos and UC-MSC-Exos (53). Recommended primary endpoints for Phase I studies include adverse event profiling, serum cytokine dynamics, and ovarian reserve biomarkers (AMH, AFC, FSH) (54). Phase II expansion should prioritize spontaneous ovulation rate, hormone normalization, and patient-reported quality of life metrics as co-primary endpoints (37). Long-term Phase III study designs must incorporate fertility outcomes (clinical pregnancy rate, live birth rate) alongside systemic safety surveillance extending beyond 5 years, acknowledging the chronic, progressive nature of POI (55).

Conclusion

Premature Ovarian Insufficiency remains a clinically formidable condition that imposes profound reproductive, endocrine, and systemic consequences on affected women. The emergence of nanoengineered exosome platforms represents a paradigm-shifting therapeutic opportunity, one that aligns the inherent biological sophistication of exosomes with the precision engineering capabilities of modern nanotechnology. Through systematic advances in cargo loading technologies, surface functionalization, hybrid nanoplatform design, and targeted ovarian delivery systems, nanoengineered exosomes offer the prospect of restoring functional ovarian capacity through multi-target molecular intervention rather than symptom suppression. The evidence base from 2022 to 2026 has substantially advanced mechanistic understanding and engineering capabilities in this field, with CRISPR-guided cargo enrichment, aptamer-based ovarian targeting, and stimuli-responsive polymer-exosome hybrids representing standout developments. Concurrently, critical challenges in GMP manufacturing, scalability, storage stability, and regulatory harmonization are receiving increasing scientific and institutional attention, with meaningful progress evident in bioreactor-based production systems and evolving international regulatory frameworks.

The path from bench to bedside demands convergence of multiple disciplines (molecular biology, materials science, clinical reproductive medicine, bioethics, and regulatory science) united around the goal of translating exosome science into meaningful therapeutic benefit. As AI-guided cargo design, personalized iPSC-derived exosome platforms, and combination regenerative strategies mature, the prospect of genuine ovarian regeneration in POI) restoring not merely hormonal balance but functional folliculogenesis and fertility (transitions from theoretical aspiration toward clinical reality. Nanoengineered exosomes stand as one of the most promising vehicles for achieving this transformative goal.

Findings

Recent studies (2022-2026) highlight nanoengineered exosome platforms as a promising regenerative therapy for Premature Ovarian Insufficiency (POI), surpassing the limitations of conventional Hormone Replacement Therapy (HRT). Advanced nanoengineering—including

targeted cargo loading, surface modifications, and hybrid delivery systems like hydrogels—significantly enhances exosome retention and targeting in the ovaries. By inhibiting granulosa cell apoptosis, reducing oxidative stress, and modulating the immune microenvironment, these targeted platforms successfully restore folliculogenesis and improve the secretion of key hormones (estrogen and AMH).

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

POI: Premature Ovarian Insufficiency
HRT: Hormone Replacement Therapy
MSCs: Mesenchymal Stem Cells
EVs: Extracellular Vesicles
GCs: Granulosa Cells
AMH: Anti-Müllerian Hormone
ROS: Reactive Oxygen Species
BMSC: Bone Marrow Mesenchymal Stem Cell
UC-MSC: Umbilical Cord Mesenchymal Stem Cell
ADSC: Adipose-Derived Stem Cell
CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
GMP: Good Manufacturing Practice
RES: Reticuloendothelial System
LNP: Lipid Nanoparticle
PLGA: Poly (lactic-co-glycolic acid)

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