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REVIEW

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Current status and future prospects of mesenchymal stem cell-derived exosomes therapy for premature ovarian insufficiency

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Abstract

Premature ovarian insufficiency (POI) is an endocrine disorder that seriously affects the reproductive health and quality of life of women of reproductive age. At present, there is no effective treatment to restore ovarian function. In recent years, accumulating studies have demonstrated that mesenchymal stem cells-derived exosomes (MSC-EXO) can restore ovarian function and fertility in POI animals by inhibiting apoptosis of ovarian granulosa cells, inflammatory response or fibrosis, and improving vascular function. However, there are still many deficiencies in this new treatment, such as the lack of standardized production of EXO, long-term safety and efficiency issues, and low homing efficiency, which limit its clinical translation and widespread application. Therefore, further research is required to address challenges such as achieving standardized mass production and targeted delivery of EXO, as well as to fully elucidate their underlying mechanisms of action. This review comprehensively summarizes the current progress of MSC-EXO in POI treatment, covering topics including MSC-EXO extraction and identification techniques, their therapeutic mechanisms in POI, POI animal models, and the clinical application potential of MSC-EXO, aiming to provide support for advancing basic research and facilitating clinical applications in this field.

Keywords Premature ovarian insufficiency, Mesenchymal stem cell, Exosomes

Introduction

Premature ovarian insufficiency (POI) refers to a decline in ovarian function in women under 40 years old, characterized primarily by abnormal menstruation, follicle stimulating hormone (FSH) > 25 U/L, and fluctuating decreased in estrogen levels [1]. It is a heterogeneous disease resulting from genetic factors, autoimmune diseases, iatrogenic factors (including chemotherapy, radiotherapy and surgical procedures) or environmental factors [2]. In addition, a large proportion of patients belong to idiopathic POI of unknown etiology. Currently, the global incidence of POI is 3.5%, which is higher than previously thought, emphasizing that POI is not a rare disease [3, 4]. Apart from idiopathic POI, iatrogenic causes and autoimmune causes are the 2 main causes of POI. At any stage of POI, female fertility can be affected by increased follicle destruction, decreased number of follicles, and failure of follicles to respond to stimulation with gonadotropins [5]. Women diagnosed with POI have a conception probability of only 5% to 10%. Moreover, POI patients are also at risk of long-term complications, including osteoporosis, fractures, cardiovascular diseases and depression [6].

The administration of estrogen and progestins in continuous or periodic regimens, termed hormone replacement therapy, can mitigate estrogen deficiency symptoms

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to a certain degree, such as alleviating menopausal symptoms like hot flashes and night sweats, and reducing the risk of osteoporosis. However, it fails to fundamentally restore ovarian function and has limited efficacy in treating infertility. Furthermore, long-term use of hormone replacement therapy may increase the risk of thromboembolism, breast cancer, ovarian cancer, dementia [1, 7, 8]. At present, oocyte donation in vitro fertilization-embryo transfer is the only effective treatment for most POI patients to achieve fertility [1, 8]. Nevertheless, the current routine clinical procedures of assisted reproductive technology do not confer broad benefits to all women undergoing in vitro fertilization treatment for POI. Embryo/oocyte cryopreservation, as a preventive strategy, also imposes a substantial financial burden and is accessible only to a small number of high-income women globally. Other therapeutic approaches, such as Chinese herbal medicine and nutritional supplements, have limited evidence supporting their effectiveness [8]. To date, no interventions have been reliably shown to enhance ovarian activity and improve natural conception rates in POI patients [8].

Accumulating preclinical and clinical evidence has demonstrated that mesenchymal stem cells (MSCs)-based therapy holds great promise and potential for the treatment of POI [9–11]. MSCs are non-hematopoietic stem cells exhibiting multi-directional differentiation potential, self-renewal ability, low immunogenicity and immunomodulatory function. These cells are easily separated from different sources, such as bone marrow, umbilical cord, adipose tissue, and etc [12]. Previous studies have demonstrated that MSCs derived from human bone marrow, adipose tissue, umbilical cord, placenta, menstrual blood can effectively restore ovarian function in POI animal models [11, 13–17]. Indeed, some clinical trials have yielded promising outcomes [9, 10, 18]. However, the clinical application of MSCs remains controversial due to several safety concerns associated with their therapeutic use, such as stem cells regulation, accidental differentiation following in vivo transplantation, cancer risk, ethical issues, heterogeneity of donor or tissue source, unclear administration frequency and special growth requirements, etc [19]. Especially in disease states, there is an urgent need to accurately estimate the appropriate number of cells for transplantation, as these cells may be affected by special conditions such as apoptosis, inflammation, and internal environment disturbance associated with POI. Therefore, the clinical application of MSCs has been significantly restricted. Subsequent studies have indicated that the therapeutic effect of MSCs is largely mediated by the delivery of exosomes (EXO) [20, 21]. Currently, studies on MSC-EXO for the treatment of POI has garnered increasing attention. MSC-EXO derived from umbilical cord, bone marrow, amniotic fluid, adipose tissue and menstrual blood have been confirmed to improve ovarian function and reduce follicular atresia, thereby providing a novel strategy and hope for POI treatment [20–24]. Through a comprehensive review of the relevant literature, this study summarizes the characteristics of MSC-EXO, extraction and identification methods, action mechanism, POI animal models and the clinical application potential of MSC-EXO, aiming to provide a more comprehensive and in-depth theoretical foundation and practical guidance for POI treatment.

Characteristics of MSC-EXO

EXO are small extracellular vesicles with a diameter of 30–150 nm and a density of 1.13–1.19 g/mL. EXO originate from the endosomal system or arise through plasma membrane shedding, and they represent membranous structures with lipid bilayer membranes. The biogenesis of EXO involves multiple stages, including plasma membrane invagination to form early endosomes, cargo sorting to generate late endosomes, and conversion of late endosomes into multivesicular bodies. Multivesicular bodies contain intraluminal vesicles. After the fusion of multivesicular bodies with the plasma membrane, EXO will be released [25–27]. (Fig. 1).

EXO contain proteins, lipids, nucleic acids. Certain tetraspanin proteins (e.g., Alix, TSG101, CD9, CD63, CD81, CD82) regulate cell adhesion and fusion [28]. Membrane proteins such as CD55 and CD59 stabilize EXO in the extracellular milieu by inhibiting the complement system [29]. They also include guanosine triphosphatases, Rab proteins (Rab11, Rab27a, Rab27b), and heat shock proteins (HSP70, HSP90) [30]. Additionally, EXO contain a large number of lipid components, including phosphatidylcholine, phosphatidylserine, and sphingolipids—key components of exosomal membranes [31]. Furthermore, EXO contain deoxyribonucleic acid (DNA, including mitochondrial DNA), messenger ribonucleic acid and micro ribonucleic acid (miR). These cargos are delivered to recipient cells *via* EXO and mediate critical functions in gene regulation. They mediate intercellular communication by regulating biological processes such as cell proliferation, apoptosis, immune regulation, metabolic regulation [32]. (Fig. 1) This EXO-mediated signaling is both efficient and stable, and compared with MSCs-based therapy, EXO offer advantages including standardized extraction process, low immunogenicity, minimal tumorigenic risk, ease of large-scale and cost-effective production, and reduced ethical concerns—attributed that render them more suitable for clinical translation than MSCs [33].

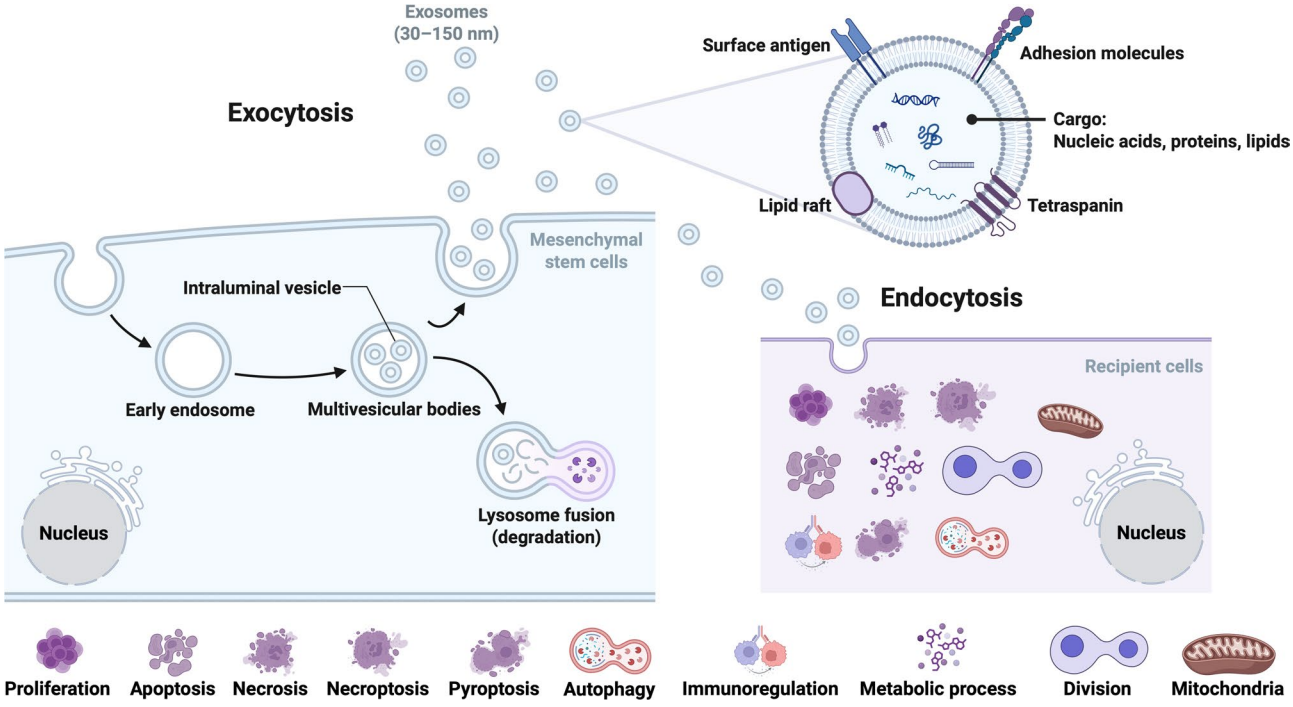


Fig. 1 The demonstration of the life journey and microscopic structure of EXO. (Created with Biorender.com)

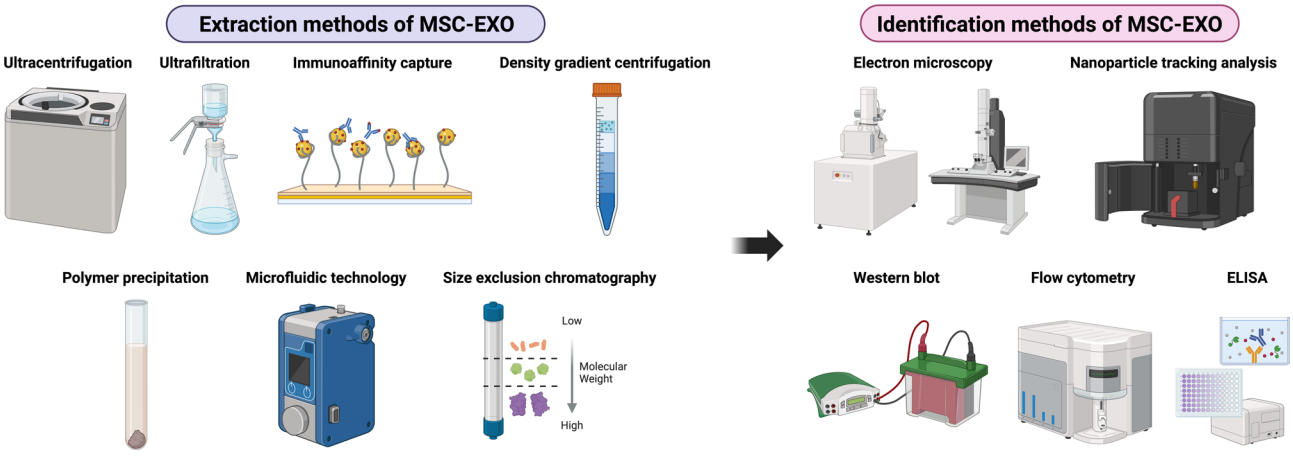


Fig. 2 Extraction and identification methods of MSC-EXO. MSC-EXO, mesenchymal stem cell-derived exosomes; ELISA, enzyme-linked immunosorbent assay. (Created with Biorender.com)

Methods for extraction and identification of MSC-EXO

The extraction and identification of MSC-EXO serve as foundational steps for investigating their biological functions and therapeutic applications, which is crucial for elucidating their action mechanism and facilitating the development of effective treatment strategies. (Fig. 2; Table 1).

Methods for extraction of MSC-EXO

Ultracentrifugation Ultracentrifugation is the most common and traditional method for EXO isolation [34]. This method relies on differences in density and sedimentation velocity between EXO and other components, achieving separation through stepwise centrifugation at varying speeds [35]. The advantages of ultracentrifugation include its relatively simple operational principle, requirement for no specialized reagents, and ability to isolate relatively large quantities of EXO. However, this method exhibits notable limitations. For example, the protocol necessitates special equipment (ultra-high-speed centrifuge) and involves multiple centrifugation steps at incremental speeds over extended periods, rendering it labor- and time- intensive. Mechanical force during centrifugation may lead to the aggregation and struc-

Table 1 The characteristics of each MSC-EXO extraction and identification method

Methods	Purposes	Principles	Advantages	Disadvantages
Ultracentrifugation	Extraction	Density and sedimentation velocity	Simple operation; reagents-independent; high yields	Time-consuming; equipment-dependent; low purity; aggregation and structural destruction of EXO
Ultrafiltration	Extraction	Molecular weight and size	Simple operation; save time; equipment-independent; preserve structural and biological integrity	Low yield; low purity
Immunoaffinity capture method	Extraction	Antigen-antibody specific binding	High specificity; high purity; preserve structural and biological integrity	High reagent costs; low yields
Density gradient centrifugation	Extraction	Sedimentation coefficient and buoyant density	High purity; preserve structural and biological integrity	Complex operation; low yields; equipment-dependent
Polymer precipitation	Extraction	Combine with the hydrophobic lipid bilayer of EXO to alter their solubility and dispersion properties	Equipment-independent; simple operation; save time; high yields	Low purity; reagents-dependent
Microfluidic technology	Extraction	Size-based filtration; inertial focusing & dean flow; antigen-antibody specific binding; dielectrophoresis; acoustic separation	Simple operation; save time; high yields; high specificity	Equipment-dependent; high equipment costs; low yields; insufficient technique standardization
Size exclusion chromatography	Extraction	Molecular size and weight	High purity; reserve structural and biological integrity	Equipment-dependent; low yields
Electron microscopy technique	Identification	Electron beam-sample interactions	Visually display the internal structure and morphological details	High equipment costs; complex operation; cannot accurately quantify EXO
Nanoparticle tracking analysis	Identification	Brownian motion and light scattering	Simple operation; accurately quantify EXO	Cannot distinguish EXO from other similarly sized nanoparticles; limited detection range
Western blot	Identification	Antigen-antibody specific binding	High specificity and sensitivity	Complex operation; require large sample volume
Flow cytometry	Identification	Molecular size and scattered light signal; antigen-antibody binding	Efficient; accurately quantify EXO	Equipment-dependent
Enzyme-linked immunosorbent assay	Identification	Antigen-antibody specific binding	Simple operation; high sensitivity and specificity	Limited detection range

tural destruction of EXO, thereby affecting the integrity and biological activity of EXO. In addition, some impurities with similar density or sedimentation rate to EXO are difficult to be separated, leading to frequent contamination of final EXO preparations with protein aggregates, lipoproteins, and cell debris—impurities that can hinder the identification of EXO-specific proteins, disrupt cellular physical responses, and compromise downstream research and applications. To mitigate these limitations, researchers can enhance EXO extraction efficiency and purity by using hierarchical centrifugation optimization, strict control of the temperature and time of the centrifugation process, or in combination with other separation methods (e.g., density gradient centrifugation and ultrafiltration) [36, 37].

Ultrafiltration The principle of ultrafiltration relies on separating sample components according to differences in their molecular weight or size. For example, selecting an ultrafiltration tube with a suitable molecular weight cutoff utilizes hydrostatic pressure to filter out low-molecular-weight substances and solvents, while retaining macromolecules like EXO-thereby achieving component separation and sample concentration. This method is simple and rapid to perform without special equipment. Additionally, low-speed centrifugation imposes low mechanical force on EXO, thereby preserving their structural and biological integrity, minimizing damage, and maintaining their natural state [38, 39]. However, the ultrafiltration method has some limitations. For instance, impurities such as proteins and cell debris tend to adhere to the ultrafiltration membrane surface, leading to membrane pollution, reducing flux, and affecting extraction efficiency and purity of EXO. Moreover, smaller EXO may be lost due to membrane interaction or pore adsorption, reducing EXO recovery. Concurrently, components of similar or larger size to EXO may co-purify with EXO, further compromising purity. Finally, variations in pore size distribution, material properties, surface charge and other aspects of ultrafiltration membranes produced by different manufacturers can directly affect EXO recovery and purity [40].

Immunoaffinity capture method The immunoaffinity capture method employed immunomagnetic beads coated with antibodies against specific exosomal surface

markers (e.g., CD9, CD63), leveraging antigen-antibody specific binding to isolate EXO [41]. This method offers high specificity, enabling effective discrimination of EXO based on their cellular origin or marker expression profile—critical for studying EXO from specific cell types or with specialized functions. Moreover, its high specificity guarantees high-purity EXO isolates, directly enhancing the accuracy and reliability of subsequent detection and analysis. In addition, this method is relatively simple and efficient, imposing low mechanical force on EXO—thereby preserving their structural and biological integrity. However, the immunoaffinity capture method also has some drawbacks, including high costs associated with antibodies and magnetic beads, as well as variability in antibody specificity and affinity that can compromise EXO extraction efficiency. Furthermore, the elution buffer employed in this method may disrupt exosomal surface protein structures, directly interfering with downstream functional analyses.

Density gradient centrifugation Density gradient centrifugation relies on differences in the sedimentation behavior of different biological particles within density gradient media (e.g., iodixanol, sucrose) to purify and isolate EXO [42]. Using sucrose as an example, sucrose/D_2O solutions with varying concentration gradients are prepared, followed by sample loading and centrifugation at 100,000 g for 2.5 h. EXO accumulate in the 1.13–1.19 g/mL isodense layer, and purified EXO are obtained by collecting the fractions within this density range. Density gradient centrifugation effectively eliminates protein and other contaminant interference, yielding EXO with superior purity and preserved structural integrity. Nonetheless, this method involves complex procedures, requiring pre-preparation of density gradient media and prolonged centrifugation times. Additionally, the intricate workflow increases the risk of sample loss or cross-contamination between different zones, affecting the extraction efficiency and purity of EXO.

Polymer precipitation Polymer precipitation is a pivotal technique in commercial EXO extraction kits, with polyethylene glycol being the most widely employed precipitation reagent. Polyethylene glycol is highly hydrophilic and can combine with the hydrophobic lipid bilayer of EXO, altering their solubility and dispersion properties and facilitating precipitation for separation [43, 44]. This method enables separation *via* conventional centrifugation alone, and the purity of EXO can be significantly enhances by incorporating a 0.22-μm filter membrane. This method is simple, rapid, and yields a high recovery rate of EXO. However, this method often results in the co-precipitation of proteins and ribonucleic acid (RNA), leading to low EXO purity and challenges in complete polymers removal, which may have adverse effects on downstream analysis [39, 45].

Microfluidic technology Microfluidic technology utilizes the microchannels and nanostructures of microfluidic chip to achieve efficient EXO separation and enrichment, offering advantages such as simple and rapid operation and less sample consumption. However, chip fabrication is complex and requires supporting microfluidic pumps, microscopes, or sensors, making integrated systems costly. Currently, this technology is still in the research stage and has not been widely adopted for practical applications, owing to lack of standardization, limited large-scale testing of clinical sample testing, and insufficient method verification [46–48].

Size exclusion chromatography Size exclusion chromatography uses gel columns to separate EXO according to their size and molecular weight, with the resulting EXO exhibiting size uniformity under electron microscopy due to the gel matrix's molecular sieve effect—though specialized equipment is necessary [49, 50]. Size exclusion chromatography can be integrated with ultracentrifugation or complementary techniques to enhance EXO yields.

Methods for identification of MSC-EXO

Electron microscopy technique Electron microscopy (including transmission electron microscopy and scanning electron microscopy) utilizes electron beam-sample interactions for imaging, enabling clear visualization of EXO morphology, size, and structure [51]. Transmission electron microscopy relies on the differential imaging of the scattering degree of electrons in different parts of the sample, thereby observing the internal structure and morphological details of EXO. EXO typically exhibit a cup- or saucer- shaped morphology, although some may appear round or oval, with diameters ranging from 30 to 150 nm. Scanning electron microscopy constructs three-dimensional image by detecting secondary electrons signals emitted from the sample, primarily for observing exosomal surface morphology and distribution. EXO generally display a relatively smooth morphology and spherical appearance, existing as single entitles or in multiple clusters. Electron microscopy is critical for confirming EXO presence and distinguish EXO from other extracellular vesicles or impurities. However, electron microscopy has notable limitations: high equipment costs, complex sample preparation, and potential structural artifacts induced by chemical treatments or physical manipulations during processing—factors that can compromise the assessment of native EXO morphology. Electron microscopy enables

qualitative observation of exosomal morphological features but cannot accurately quantify EXO.

Nanoparticle tracking analysis Nanoparticle tracking analysis technology enables rapid and precise measurement of EXO size distribution and concentration by leveraging Brownian motion and light scattering principles [52, 53]. When the laser illuminates EXO in solution, the scattered light signals are captured by a detector, and EXO size and concentration are calculated from scattered light intensity and particle Brownian motion velocity. Nanoparticle tracking analysis is operationally simple, with sample preparation and detection process, and it enables intuitive and accurate size and concentration analysis that reflects the native state of EXO. However, nanoparticle tracking analysis has some limitations: it cannot distinguish EXO from other similarly sized nanoparticles, thus necessitating integration with complementary methods for sample identification. In addition, its detection range is typically 10 – 1,000 nm, and the presence of abundant particles outside this range can compromise measurement accuracy.

Western blot Western blot, grounded in specific antigen-antibody binding, serves to identify exosomal surface markers (e.g., CD9, HSP70), thereby enabling the determination of EXO presence and relative abundance [54]. Proteins within EXO are first separated by polyacrylamide gel electrophoresis, transferred to a solid phase membrane, and detected by specific antibodies to determine EXO presence in samples. Semi-quantitative analysis *via* band intensity comparison reveals relative differences in exosomal protein expression across samples, facilitating the investigation of EXO changes under various physiological or pathological conditions. Western blot exhibits high specificity and sensitivity, enabling simultaneous detection of multiple exosomal markers. However, this technique involves complex procedures, with experimental accuracy highly contingent on antibody quality and requiring relatively large sample volume.

Others Additional identification techniques include flow cytometry and enzyme-linked immunosorbent assay [55]. Flow cytometry enables quantitative analysis of exosomal surface markers and characterization of size, physical parameters, and morphology. However, the nanoscale size of EXO necessitates specialized instruments and techniques to enhance detection accuracy. Enzyme-linked immunosorbent assay primarily detects the abundance of specific exosomal proteins, offering advantages such as operational simplicity and high sensitivity. However, enzyme-linked immunosorbent assay is restricted to detecting known proteins, exhibiting limited capability for unknown analytes.

Potential mechanisms of different tissue-derived MSC-EXO in the treatment of POI

Research has demonstrated the efficacy of various MSC-EXO in promoting ovarian function, laying a foundation for their potential use in POI therapy. These effects include inhibiting apoptosis, autophagy and pyroptosis of ovarian granulosa cells, promoting cell proliferation, inhibiting the production of reactive oxygen species and oxidative stress response, inhibiting inflammatory response, promoting ovarian angiogenesis, and inhibiting ovarian tissue fibrosis (Fig. 3). The following sections will elaborate on these mechanisms in detail.

Human umbilical cord MSC-EXO (hUCMSC-EXO)

HUCMSCs are a subset of MSCs derived from neonatal umbilical cord tissue, primarily isolated from Wharton's jelly, umbilical cord perivascular tissue, or umbilical cord blood [18]. Owing to their ease of collection, strong proliferation ability and low immunogenicity, hUCMSCs-EXO are frequently utilized in regenerative medicine and the treatment of various diseases. Among studies on MSC-EXO for POI treatment, those focusing on hUMSC-EXO are the most numerous, and all have reported favorable therapeutic outcomes. Therefore, hUCMSC-EXO represent a promising candidate drug for preserving ovarian reserve and improving ovarian function. HUCMSC-EXO may treat POI through multiple mechanisms, such as inhibiting apoptosis and autophagy of ovarian granulosa cells, promoting cell proliferation, suppressing the production of reactive oxygen species and oxidative stress response, inhibiting inflammatory response, promoting ovarian angiogenesis, and inhibiting ovarian tissue fibrosis. (Fig. S1).

Studies have demonstrated that hUCMSC-EXO can mitigate oxidative stress and apoptosis of ovarian granulosa cells [56, 57], and this effect may be attributed to specific miRs abundant in hUCMSC-EXO. For example, miR-17-5p in hUCMSC-EXO inhibits SIRT7-PARP1/γH2AX/XRCC6 axis [58]; miR-145-5p targets XBP1 [59]; miR-29a down-regulates HBP1 and activates the Wnt/β-catenin pathway [60]; miR-126-3p regulates the PI3K/AKT/mTOR signaling pathway by targeting PIK3R2 [61]; miR-22-3p targets KLF6 and the ATF4-ATF3-CHOP pathway [62]; miR-20b-5p down-regulates PTEN and regulates the PI3K/AKT pathway [63]. These specific miRs all have been proven to promote the proliferation of ovarian granulosa cells, suppress apoptosis, and mitigate chemotherapy-induced ovarian damage. Moreover, hUCMSC-EXO can also achieve the same therapeutic effect by inhibiting ferroptosis through the Nrf2/GPX4 signaling pathway [64]. In addition to miRs, certain circRNAs in hUCMSC-EXO may also be involved in the anti-apoptotic process. For instance, circ_0002021, acting as a competitive sponge for miR-125a-5p, can regulate CDK6

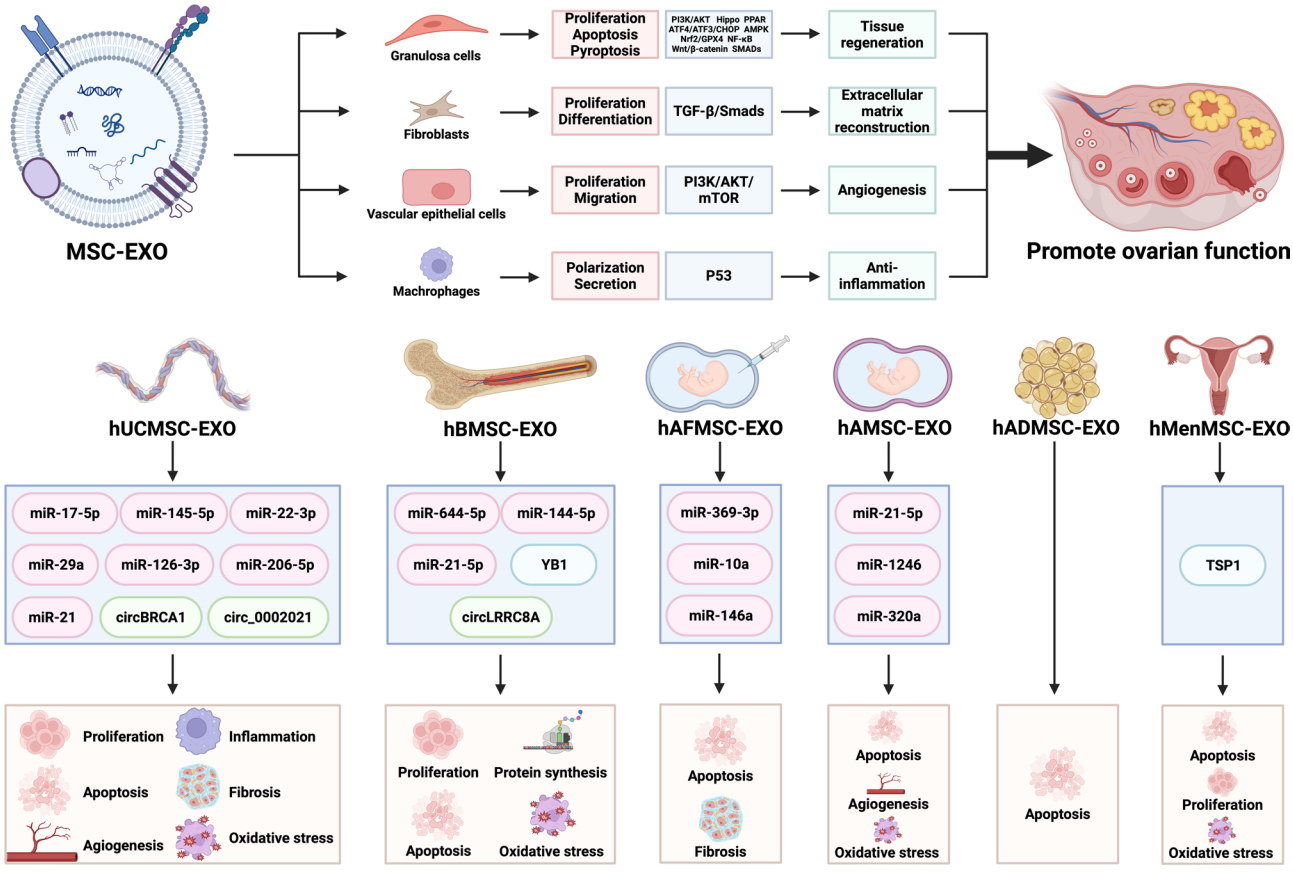


Fig. 3 Summary of active components and therapeutic effects of MSC-EXO from various tissues in POI treatment. POI, premature ovarian insufficiency; MSC-EXO, mesenchymal stem cell-derived exosomes; hUCMSCs, human umbilical cord mesenchymal stem cells; hBMSCs, human bone marrow mesenchymal stem cells; hAFMSCs, human amniotic fluid mesenchymal stem cells; hAMSCs, human amniotic mesenchymal stem cells; hADMSC-EXO, human adipose mesenchymal stem cell-derived exosomes; hMenMSCs, human menstrual blood mesenchymal stem cells; miRs, micro ribonucleic acid; circ, circular; TSP1, thrombospondin-1. (Created with Biorender.com)

expression and improve the senescence state of ovarian granulosa cells [65]. M6A-modified circBRCA1 can directly sponge miR-642a-5p, subsequently up-regulate FOXO1, resisting oxidative stress damage of granulosa cells, and protecting the ovarian function of POI rats [66]. CircDennd2a also effectively counteracts granulosa cells apoptosis by enhancing the glycolytic process and promoting cell proliferation [67].

In addition to inhibiting ovarian granulosa cells apoptosis, hUCMSC-EXO can also improve ovarian function by suppressing the inflammatory response in ovarian tissues. Some studies have indicated that intravenous injection of hUCMSC-EXO can improve the inflammatory environment within the ovary, reduce the proportion of M1-like macrophages, and significantly decrease the expression of pro-inflammatory factors. Its potential mechanism may be related to the anti-inflammatory function of p53 [68]. Furthermore, angiogenesis also pivotal in the application of hUCMSC-EXO. Yang et al. demonstrated that hUCMSC-EXO activate the PI3K/AKT pathway and promote VEGF expression, thereby facilitating angiogenesis and the recovery of damaged ovaries [69]. Subsequent research by Qu et al. revealed that this effect may be achieved through the delivery of miR-205-5p targeting PTEN [70] or miR-126-3p targeting PIK3R2 [61]. Moreover, miR-21 in hUCMSC-EXO down-regulates LATS1, thereby reducing phosphorylated LOXL2 and YAP and ultimately promoting estrogen secretion of ovarian granulosa cells and restoring ovarian function [71]. Apart from their effects on granulosa cells, hUCMSC-EXO can attenuate ovarian tissues fibrosis by inhibiting the TGF-β1/Smad3 signaling pathway. They can significantly inhibit the expression of the fibrosis marker α-SMA and the production of type I and type III collagens, and enhance the differentiation of follicular cells, thereby contributing to the recovery of ovarian function [72].

Human bone marrow MSC-EXO (hBMSC-EXO)

HBMSCs represent a subpopulation of multipotent cells and are a focal point in stem cell therapy, typically harvested from the anterior superior iliac spine

and posterior superior iliac spine [73, 74]. HBMSCs are characterized by easy accessibility, strong proliferative capacity, high plasticity, and strong adherence properties [74]. The mechanisms by which hBMSC-EXO improve ovarian function may include inhibiting apoptosis and pyroptosis of ovarian granulosa cells, as well as alleviating oxidative damage. (Fig. S2) Studies have revealed that hBMSC-EXO activate the PI3K/AKT pathway by delivering miR-144-5p to inhibit PTEN [24]; they can also down-regulate PTEN and PDCD4 by delivering miR-21-5p [75], and down-regulate p53 by delivering miR-644-5p [76], thereby inhibiting the ovarian granulosa cell apoptosis. Beyond their therapeutic efficacy in chemotherapy-induced POI mice, hBMSC-EXO can also treat autoimmune POI mice by mediating MSX1 to regulate the Notch [77] or the MALT1/NF-κB [78] or the NLRP3/Casp1/GSDMD signaling pathway [79]. Moreover, hBMSC-EXO can promote the proliferation of ovarian granulosa cells and hormone synthesis in vitro, while inhibiting cell apoptosis and pyroptosis. Furthermore, hBMSC-EXO may enhance ovarian function through the release of the specific protein YB-1. YB-1 has been demonstrated to be a cellular stress response factor, crucial for inhibiting premature cell exhaustion in vitro and regarded as a promising candidate molecule for regulating cell senescence [80, 81]. Lu et al. found that YB-1 downregulation was detected in both the ovarian tissues of chemotherapy-induced POI rats and the hydrogen peroxide-induced POI cell model, and hBMSC-EXO treatment reversed YB-1 protein expression. Research has confirmed that hBMSC-EXO can mediate the regulation of the MALAT1/miR-211-5p/FOXO3 axis by delivering YB-1 [82]. In addition to miRs and proteins, circLRRC8A in hBMSC-EXO can act as an endogenous miR-125a-3p sponge to down-regulate NFE2L1 expression, subsequently exerting functions such as antioxidant stress response, metabolic regulation, endoplasmic reticulum stress response modulation, and intracellular environment maintenance. The delivery of circLRRC8A-rich EXO offers a novel therapeutic strategy for resisting oxidative damage in ovarian tissues [83].

Human amniotic fluid MSC-EXO (hAFMSC-EXO)

HAFMSCs are typically derived from amniotic fluid samples collected during diagnostic amniocentesis [84]. HAFMSC-EXO can regulate the signaling pathways of ovarian granulosa cells by delivering miRs, thereby inhibiting cell apoptosis. They can also suppress ovarian fibrosis and improve ovarian function. (Fig. S3) HAFMSC-EXO inhibit the apoptosis of damaged ovarian granulosa cells by delivering miR-146a and miR-10a, and prevent follicular atresia in mice after chemotherapy-induced damage, with miR-10a playing a predominant role [85]. MiR-10a directly regulates Bim, resulting in the downregulation of Caspase-9, a key factor in the cell apoptosis pathway. Although miR-146a down-regulates the expression of its targets Irak1 and Traf632, the complex interactions between these genes and cell apoptosis remain elusive, and its effect on cell apoptosis is less significant than that of miR-10a. Therefore, miR-146a may only play a secondary role in the treatment with hAFMSC-EXO. Moreover, CD44+/CD105 + hAFMSC-EXO carrying miR-369-3p can specifically down-regulate YAF2 expression, disrupt the stability of PDCD5/p53, and reduce the apoptosis of ovarian granulosa cells, thereby exerting a therapeutic effect on POI [86]. Furthermore, treatment with hAFMSC-EXO can significantly reduce the deposition of ovarian collagen fibers, inhibit ovarian fibrosis, and facilitate the restoration of the normal structure and function of the ovaries. This effect may be achieved through the TGF-β/Smads signaling pathway [87].

Human amniotic MSC-EXO (hAMSC-EXO)

HAMSCs are a type of stem cell isolated from the amniotic tissue adjacent to the fetal side of the placenta [88]. They exhibit a range of advantageous characteristics, including immunomodulatory, anti-inflammatory properties, non-invasive sample collection, minimal ethical concerns, superior attachment and proliferation rates, non-tumorigenicity, enhanced subculture potential, freedom from age-related heterogeneity, and an intact immune privileged phenotype – qualities that position them as crucial research subjects in clinical regenerative medicine and tissue engineering [88]. HAMSC-EXO can reduce reactive oxygen species levels in ovarian tissues, inhibit ovarian granulosa cell apoptosis, and prevent acute vascular injury by delivering miRs. (Fig. S3) Previous studies have revealed that miR-320a released from hAMSC-EXO reduces reactive oxygen species production in the ovarian tissues of POI animals by down-regulating SIRT4 expression and its related genes ANT2, AMPK, and L-OPA1, thereby increasing follicle number, restoring hormone levels, and repairing damaged ovaries [89]. Moreover, Zhang et al. demonstrated that treatment with hAMSC-EXO protects the ovaries against chemotherapy-induced apoptosis of ovarian granulosa cells and acute vascular injury by regulating metabolic pathways, including the PPAR and AMPK signaling pathway. The therapeutic mechanism may be associated with miR-1246 and miR-21-5p encapsulated within hAMSC-EXO [90].

Human adipose MSC-EXO (hADMSC-EXO)

HADMSCs are a subset of stem cells isolated from adipose tissue, characterized by multipotent differentiation potential [91]. They exhibit extensive application prospects in fields including regenerative medicine and tissue

engineering. HADMSC-EXO can significantly increase the number of follicles at all developmental stages in chemotherapy-induced POI mice, restore sex hormone levels to normal, and potently inhibit ovarian granulosa cell apoptosis. This effect may be achieved by up-regulating the expression of SMAD2, SMAD3 and SMAD5, thereby decreasing the expression of Fas, FasL, Caspase-3, and Caspase-8 [92]. (Fig. S3) In addition, combined treatment with the drug-free in vitro activation method of the ovary can exert a more remarkable therapeutic effect on POI [93].

Human menstrual blood MSC-EXO (hMenMSC-EXO)

During menstruation, as the functional layer of the endometrium disintegrates and sloughs off, a large number of endometrial MSCs with regenerative potential are shed into the menstrual blood along with tissue debris. These cells-referred to as hMenMSCs-can be isolated from endometrial fragments in menstrual blood using a specific collagenase digestion protocol [94]. Characterized by non-invasive procurement, high proliferation ability, and absence of ethical concerns, they represent an ideal tissue source for generating MSC-EXO. Zhang et al. demonstrated that hMenMSC-EXO could significantly promote follicular development both in vivo and in vitro, improve ovarian function, and restore the fertility of POI rats. Notably, there was no significant difference in therapeutic efficacy between hMenMSC-EXO treatment and hMenMSCs treatment [20]. Subsequently, Song et al. found that thrombospondin-1 (TSP1) was an important functional component of hMenMSC-EXO in the treatment of POI [95]. TSP1 is a multifunctional extracellular matrix glycoproteins that participates in various physiological and pathological processes, including cell signaling, wound healing, cell adhesion, and angiogenesis [96]. HMenMSC-EXO partially mediated the therapeutic effect on ovarian function in the POI rat model by delivering TSP1, and promoted the proliferation and inhibited the apoptosis of 4-vinylcyclohexene dicycloxide (VCD)-induced POI cells by activating the SMAD3/AKT/p53/MDM2 signaling pathway [95]. In addition, Marinaro et al. found that hMenMSC-EXO could improve the quality and quantity of embryos in an aged mouse model. This effect was achieved *via* the regulation of antioxidant enzymes and promotion of pluripotent activity [97]. (Fig. S3).

Animal models and applications of MSC-EXO in the treatment of POI

Currently, research into the use of MSC-EXO for treating POI remains in the preclinical stage, and no clinical trials have been conducted thus far to investigate the therapeutic efficacy of MSC-EXO in POI patients. Therefore, the successful establishment of various POI animal models is crucial for evaluating the therapeutic effect and safety of MSC-EXO in POI with different etiologies. The POI animal models applied in published research on the treatment of POI with MSC-EXO include the cyclophosphamide (CTX)-induced POI model, the cisplatin (CIS)-induced POI model, the VCD-induced POI model, and the zona pellucida (ZP) glycoprotein 3-induced POI model. (Fig. 4; Table 2) The etiologies of POI associated with the aforementioned modeling methods include chemotherapy, autoimmunity, environmental factors, etc., with the chemotherapeutic drug-induced POI model being the most prevalently used. Studies have revealed that MSC-EXO can significantly improve the ovarian function of the aforementioned POI animal models or mitigate the detrimental effects of drugs on ovarian function [20, 21, 24, 58–62, 64–66, 76, 77, 79, 82, 83, 85–87, 92, 93, 95, 98–101].

The POI animal model induced by CTX

CTX is a widely utilized chemotherapy agent renowned for its both broad-spectrum anti-tumor efficacy and immunosuppressive properties. Clinically, it is frequently employed in the treatment of ovarian cancer, breast cancer, leukemia, malignant lymphoma, autoimmune diseases, etc. However, CTX exhibits serious reproductive toxic side effects and can cause permanent damage on gonadal tissue. When establishing a POI model, CTX can be used either alone or in combination with busulfan, both of which can induce ovarian tissue damage and functional loss. In currently published studies, the primary route of CTX administration for establishing the POI model is intraperitoneal injection; however, the dosage and injection interval vary. The most frequently used dosage regimen is an initial injection of 50 mg/kg, followed by daily injections of 8 mg/kg for 13 consecutive days [65, 82, 83, 92, 93]. However, Dai et al. compared the success rates of POI model establishment using alone or in combination with busulfan *via* different injection methods [99]. The results demonstrated that ultrasound-guided intra-ovarian injection of CTX (50 μg/gram of ovarian weight, with the same dose reinjected after a 2-week interval) yielded a higher model establishment success rate compared to intraperitoneal injection of CTX alone or in combination with busulfan. Moreover, compared with systemic injection of chemotherapeutic drugs, local injection exhibits greater ovarian toxicity, induces fewer systemic complications (such as bone marrow suppression and bleeding), and consequently, the body demonstrates stronger tolerance [75]. This approach can effectively prevent multi-organ damage and results in a lower mortality rate. However, in-situ ovarian injection requires ultrasound equipment and professionals with extensive experience in ultrasound-guided ovarian injection techniques, rendering the procedure

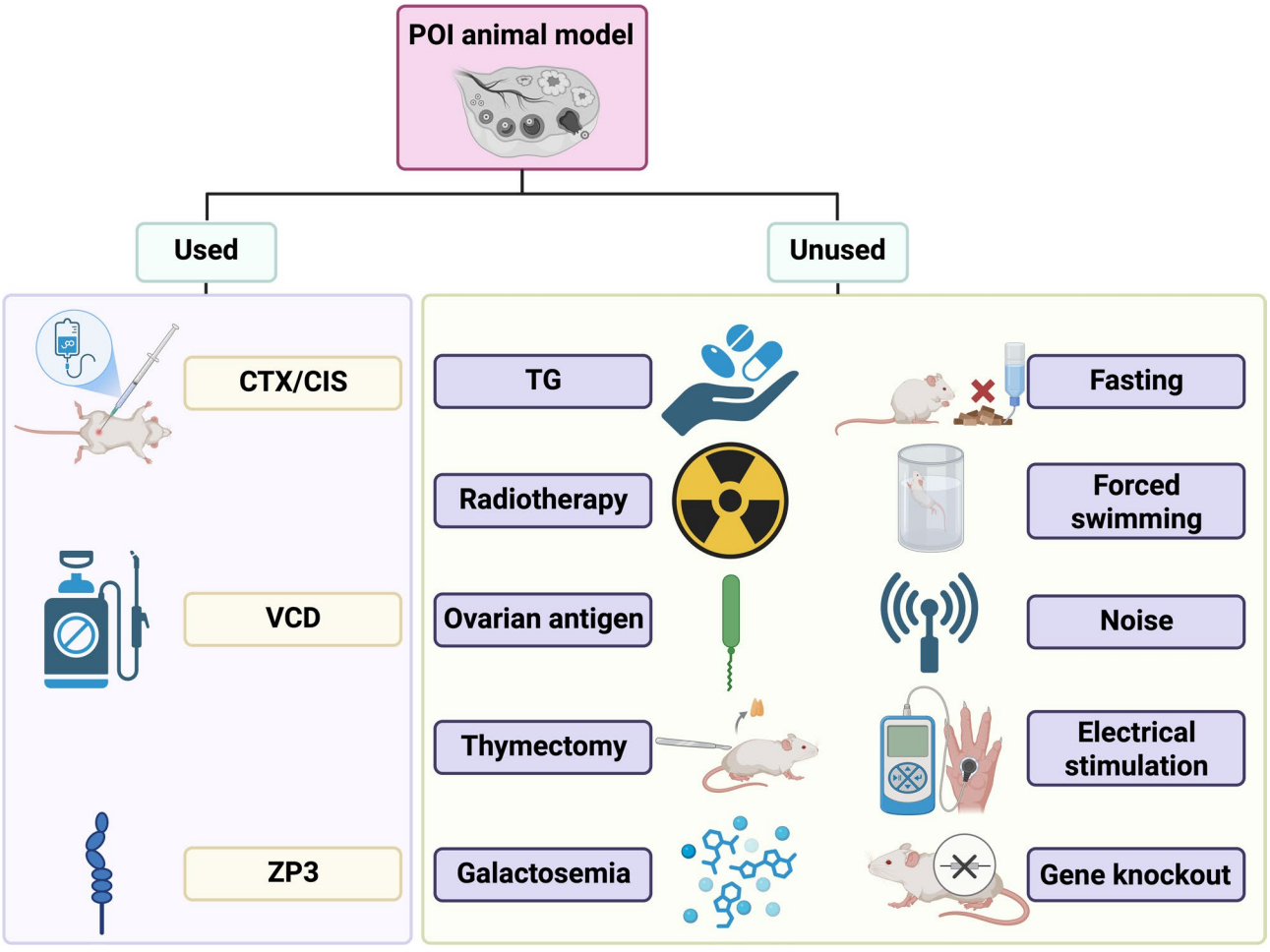


Fig. 4 Schematic diagram of the summary of premature ovarian insufficiency animal models. POI, premature ovarian insufficiency; CTX, cyclophosphamide; CIS, cisplatin; ZP3, zona pellucida glycoprotein 3; TG, tripterygium glycosides. (Created with Biorender.com)

complex. Therefore, this method has not been widely adopted to date.

The POI animal model induced by CIS

CIS primarily induces ovarian damage by activating cellular apoptosis and oxidative stress responses. The injection route of CIS is typically intraperitoneal injection as well. The dosages employed in current studies mainly consist of administering 1 mg/kg or 2.5 mg/kg for 14 consecutive days [61, 62]. The CIS-induced POI animal model boasts advantages such as simple operation, low cost, short experimental cycle, low mortality rate, and high reproducibility. It also exhibits pathological mechanisms and tissue characteristics similar to those of the ovaries affected by clinical chemotherapy-induced POI. Therefore, it is also a commonly adopted approach for model establishment when exploring the pathology and treatment of POI. However, similar to CTX, the injection of CIS may inflict non-specific damage on other organs and tissues, affecting the overall health and physiological condition of the experimental animals, potentially interfering with the experimental outcomes.

The POI animal model induced by VCD

VCD, a prevalent industrial chemical and environmental pollutant, is widely present in daily products like rubber items, insecticides, and fragrances, exhibiting potent reproductive toxicity. Flaws et al. demonstrated that VCD damages oocytes in the ovaries of mature rats, with small follicles being particularly susceptible to its toxic effects. It may reduce overall survival by impairing the viability of granulosa cells within oocytes [102, 103]. Wei et al. found that VCD induces follicular atresia by promoting apoptosis of ovarian granulosa cells [104]. Therefore, VCD serves as an ideal and classic chemical reagent for establishing POI models. Currently, the standard protocol for VCD-induced POI model establishment involves intraperitoneal injection of 80 mg/kg/d for 15 consecutive days [20, 95].

Table 2 Animal models used in the research direction of EXO therapy for POI

Mode of modeling	Drug of modeling	Injection method	Injection frequency and dosage	Animals of modeling	Tissue origin of MSC-EXO	Therapeutic effects	References
Chemotherapy	CTX	IP	A total of 2 doses of 120 mg/kg once every 7 days	C57BL/6 (8 w)	UC	ovarian morphology, follicle count, hormone levels, reproductive capacity	[21]
Chemotherapy	CTX	IP	50 mg/kg for the 1st time followed by 8 mg/kg daily for the next 13 days	C57BL/6 (6–8 w)	AT	ovarian weight, ovarian morphology, follicle count, hormone levels, oxidative stress	[100]
Chemotherapy	CTX	IP	Each dose was 50 mg/kg for 14 consecutive days	C57BL/6 (6 w)	UC	hormone levels, follicular development, ovarian weight	[64]
Chemotherapy	CTX	IP	50 mg/kg for the 1st time followed by 8 mg/kg daily for the next 13 days	SD (5 w)	BM	ovarian morphology, follicle count, hormone levels, reproductive capacity	[82]
Chemotherapy	CTX	IP	200 mg/kg for the 1st time followed by 8 mg/kg daily for the next 13 days	Wistar (50–60 g)	UC	ovarian morphology, estrous cycle, hormone levels	[59]
Chemotherapy	CTX	IP	50 mg/kg for the 1st time followed by 8 mg/kg daily for the next 13 days	SD (8–10 w)	AT	follicle count, hormone levels	[92]
Chemotherapy	CTX	IP	8 mg/kg for the 1st time followed by 2 mg/kg daily for the next 13 days	SD (5 w)	UC	ovarian morphology, estrous cycle, reproductive capacity, oxidative stress, cellular senescence	[66]
Chemotherapy	CTX	IP	50 mg/kg for the 1st time followed by 8 mg/kg daily for the next 13 days	SD (8–10 w)	AT	follicular development, hormone levels, weight, estrous cycle	[93]
Chemotherapy	CTX	IP	50 mg/kg for the 1st time followed by 8 mg/kg daily for the next 13 days	SD (5 w)	BM	ovarian morphology, estrous cycle, hormone levels, follicular development	[83]
Chemotherapy	CTX	IP	200 mg/kg for the 1st time followed by 8 mg/kg daily for the next 13 days	Wistar (7–8 w)	AF	ovarian morphology, follicle count, hormone levels, reproductive capacity, fibrosis	[87]
Chemotherapy	CTX	IP	50 mg/kg for the 1st time followed by 8 mg/kg daily for the next 13 days	C57BL/6 (6–8 w)	UC	ovarian morphology, hormone levels, oxidative stress, cellular senescence, DNA damage	[65]
Chemotherapy	CTX	IP	A single dose of 120 mg/kg	C57BL/6 (10 w)	UC	ovarian morphology, hormone levels, reproductive capacity, cell proliferation, oxidative stress, cell apoptosis	[58]
Chemotherapy	CTX	IP	70 mg/kg/d for 7 days followed by 30 mg/kg/d for 14 days	C57BL/6 (10 w)	AF	ovarian weight, follicle count, hormone levels, cell proliferation, cell apoptosis	[86]
Chemotherapy	CTX	IP	200 mg/kg for the 1st time followed by 8 mg/kg daily for the next 13 days	SD (5 w)	BM	follicle count, estrous cycle, hormone levels	[24]
Chemotherapy	CTX + busulfan	IP	CTX (120 mg/kg/d) + busulfan (30 mg/kg) for 7 days	C57BL/6	BM	follicle count, estrous cycle, hormone levels, reproductive capacity, cell apoptosis, fibrosis	[98]
Chemotherapy	CTX + busulfan	IP	CTX (120 mg/kg/d) + busulfan (30 mg/kg) for 7 days	Mice	BM	estrous cycle, hormone levels, folliculogenesis, cell apoptosis, fibrosis	[101]
Chemotherapy	CTX + busulfan	IP	A single dose of CTX (200 mg/kg) + busulfan (20 mg/kg)	ICR mice (6 w)	AF	folliculogenesis, cell apoptosis, follicular atresia	[85]

Table 2 (continued)

Mode of modeling	Drug of modeling	Injection method	Injection frequency and dosage	Animals of modeling	Tissue origin of MSC-EXO	Therapeutic effects	References
Chemotherapy	CTX	ISI	Two injections of 50 μg/gram of ovarian weight once every 14 days	Wistar (60 d)	UC	follicle count, estrous cycle, hormone levels, reproductive capacity, cell apoptosis	[99]
Chemotherapy	CIS	IP	Each dose of 1 mg/kg for 14 consecutive days	Wistar (5 w)	UC	ovarian weight, follicle count, hormone levels, angiogenesis, cell apoptosis	[61]
Chemotherapy	CIS	IP	A single dose of 5 mg/kg	C57BL/6 (6–7 w)	BM	ovarian morphology, follicle count, hormone levels, cell apoptosis	[76]
Chemotherapy	CIS	IP	A single dose of 5 mg/kg	C57BL/6 (8 w)	UC	follicle count, hormone levels	[60]
Chemotherapy	CIS	IP	Each dose of 2.5 mg/kg for 14 consecutive days	C57BL/6 (8 w)	UC	follicle count, hormone levels, cell apoptosis	[62]
Environmental	VCD	IP	Each dose of 80 mg/kg for 15 consecutive days	SD (6 w)	MenB	follicle count, ovarian weight, estrous cycle, reproductive capacity, cell proliferation, cell apoptosis	[95]
Environmental	VCD	IP	Each dose of 80 mg/kg for 15 consecutive days	SD (7 w)	MenB	follicular development, estrous cycle, hormone levels, reproductive capacity, cell proliferation	[20]
Autoimmune	ZP3	HI	Two injections of 75 μg once every 14 days	BALB/c (5–6 w)	BM	ovarian morphology, estrous cycle, hormone levels, cell proliferation, cell apoptosis, inflammatory response	[77]
Autoimmune	ZP3	HI	Two injections of 75 μg once every 14 days	BALB/c	BM	follicle count, estrous cycle, hormone levels, reproductive capacity, pyroptosis	[79]

Note: CTX: cyclophosphamide; CIS: cisplatin; VCD: 4-vinylcyclohexene dicycloxide; ZP3: Zona pellucida glycoprotein 3; IP: intraperitoneal injection; ISI: in situ injection; HI: hypodermic injection; UC: umbilical cord; AT: adipose tissue; BM: bone marrow; AF: amniotic fluid; DNA: deoxyribonucleic acid; MenB: menstrual blood. The treatment effects section of the table is in red and green font.

The POI animal model induced by ZP3

ZP3 is a critical zona pellucida glycoprotein and a sperm receptor. Injecting ZP3 glycoprotein into animals induces oophoritis *via* activation T cells mediated immune responses. Moreover, IgG antibodies against human recombinant ZP3 mediate follicular destruction and disrupt cellular communication between granulosa cells and oocytes. Therefore, ZP3-induced immune responses cause ovarian atrophy, follicular atresia, and other pathological changes. For the first immunization, 0.15 mL of complete immunoadjuvant containing 75 μg of ZP3 peptide is subcutaneously injected into the hind feet and abdomen of the mouse. Fourteen days after the first immunization, 0.15 mL of incomplete immunoadjuvant containing 75 μg of ZP3 peptide is subcutaneously injected at the same site [77, 79]. Six weeks after the second immunization, the mice exhibit irregular or prolonged estrous cycles, which indicates successful establishment of the autoimmune POI mouse model [105]. The method of inducing autoimmune POI mice with ZP3 is simple to operate, featuring a high survival rate and model success rate. This model recapitulates the pathological process of human autoimmune POI, thereby facilitating exploration of its pathogenesis and pathological changes, as well as verification of therapeutic interventions for this condition [106].

POI animal models not used in MSC-EXO related research

The etiologies of POI are complex and diverse. In addition to the above-mentioned chemical reagents (e.g., CTX, CIS, VCD) or immunological factors (e.g., ZP3) or environmental factors (e.g., VCD) used for POI model establishment, radiation and other chemical substances can also induce ovarian function failure. For other POI etiologies—including mental, metabolic, and genetic factors–relatively mature modeling methods also have been established. (Fig. 4) However, these modeling methods have not yet been applied in studies investigating the therapeutic effects of MSC-EXO. Whether MSC-EXO can ameliorate POI caused by these etiologies therefore warrant further investigation.

Tripterygium glycosides (TG) TG, a traditional Chinese patent medicine, is commonly used in treating auto-

immune diseases and rheumatoid arthritis but exhibits gonadal toxicity. TG induces DNA base mismatch and strand breakage, thereby causing a decline in ovarian estrogen secretion and an increase in FSH. Yuan et al. orally administered a 40 mg/kg/day TG suspension *via* gavage to 8-week-old female BLAB/c mice for 14 consecutive days. The mice showed characteristic changes indicative of ovarian damage [107]. The TG-induced POI animal model offers advantages such as safe oral administration and low mortality rate [108].

Radiotherapy In patients undergoing pelvic radiotherapy, the prevalence of POI reaches up to 80–100%. Radiotherapy-induced ovarian tissue damage primarily elicits apoptosis of follicles at all developmental stages, thereby causing a profound and irreversible decline in ovarian reserve function. For example, Gao et al. irradiated Kunming mice with a single dose of 4 Gy X-rays at an intensity of 100 cGy/min. The resulting POI mice showed a decrease in primordial and preantral follicle counts, an increase in atretic follicle numbers, and diminished granulosa cell proliferative capacity [109]. Tan et al. irradiated C57BL/6J mice with a single dose of X-rays at an intensity of 4 Gy and 1.2 Gy/min. Two to seven days post-irradiation, the mice exhibited signs including estrous cycle irregularities, ovarian atrophy, and reduced ovarian reserve function [110].

Autoimmune POI animal model In addition to using ZP3, ovarian antigens or thymectomy can be used to establish autoimmune POI animal models. Rat ovarian tissue supernatant protein is mixed with Freund's adjuvant to serve as the ovarian antigen. Subcutaneous injecting of 0.35 mL of this ovarian antigen 3 times every 10-day intervals successfully generates an autoimmune POI rat model [111]. Due to its limited use, the stability and success rate of this modeling method require further validation. Additionally, thymectomy in 3-day-old neonatal mice induces autoimmune oophoritis, leading to complete loss of ovarian oocytes upon adulthood [112]. However, neonatal thymectomy is technically challenging and associated with a very high mortality rate.

POI animal model induced by mental factors Mental stress, including chronic anxiety, depression, sadness, and fear, disrupts the hypothalamic-pituitary-ovarian axis and systemic immune function, thereby damaging ovarian function. Dysregulation of the hypothalamic-pituitary-ovarian axis feedback mechanism disrupts the neuroendocrine-immune biomolecular network balance, ultimately culminating in POI. Dai et al. sought to simulate early-life stress by separating mother rats from their pups on postnatal day 9, thereby inducing ovarian functional decline [99]. However, while this method demonstrated mild stress and low mortality, it failed to recapitulate key POI features, including ovarian follicles alterations and hormone level changes. This method could not effectively simulate the manifestations of POI. Therefore, researchers did not subsequently utilize this model to verify the therapeutic efficacy of MSC-EXO. In fact, stress-induced POI animal model can be constructed using alternating acoustic, light, and electric stimuli at varying frequencies [113]. A chronic unpredictable mild stress model can be established through daily alternating fasting and water deprivation, forced swimming, noise interference, and plantar electric stimulation for 35 days. Chronic mild stress elevates mental stress levels in female rats and reduces ovarian reserve [114]. This modeling method aligns with known major etiologies of human POI, with pathogenic pathways and pathological changes analogous to clinical observations. As social stressors increase, the prevalence of mental stress-induced POI rises progressively. Therefore, it is particularly important to evaluate the therapeutic effect of MSC-EXO on POI caused by mental stress.

POI animal model induced by galactosemia Galactosemia is an autosomal recessive genetic disorder caused by a deficiency of galactose-1-phosphate uridylyl transferase. Due to the systemic accumulation of galactose and its toxic metabolites, affected individuals develop progressive damage to the liver, kidneys, eyes, nervous system, and reproductive organs. Reproductive system involvement typically presents as POI or primary amenorrhea. Pregnant rats are fed a diet supplemented with 35% galactose from day 3 of gestation through lactation. Offspring exhibit ovarian dysfunction upon reaching adulthood, characterized by delayed puberty development, and eventually progress to a hypoestrogenic state with hypergonadism, thereby establishing a metabolic factor-induced POI model [115].

POI animal model induced by genetic factors Genetic knockout models have emerged as a prevalent approach for investigating gene structure-function relationships and developing human disease models. Currently, POI models can be established through genetic knockout of genes (e.g., PTEN, GDF9, BMP15) in mice [6, 116–118]. This method facilitates the elucidation of specific gene roles POI pathogenesis and progression. However, each model can only investigate 1–2 genes. Moreover, this technology entails high technical complexity, substantial costs, and stringent requirements for mouse husbandry. Therefore, broad implementation of this technology remains challenging.

Challenges faced by MSC-EXO in the treatment of POI

Although MSC-EXO have demonstrated significant therapeutic efficacy in POI animal models, several critical

challenges must be addressed before their clinical translation for POI treatment. Key challenges include the production and standardization of EXO, long-term safety and efficiency issues, and low homing efficiency.

Production and standardization of EXO

Currently, challenges including low yield, poor purity, and quality fluctuations in MSC-EXO production technology severely hinder their clinical translation [119]. The relatively low yield of MSC-EXO, combined with strict limitations on donor cell quantity and passage number, renders even industrialized cell expansion strategies insufficient to meet large-scale clinical demands. For example, a clinical trial requires 5–20 μg of MSCs per participant, administered twice weekly for 12 weeks (ClinicalTrials.gov Identifier: NCT04388982). Calculated in this way, the amount of EXO required for just 9 participants would take weeks to produce. Therefore, it is necessary to develop new methods to increase the production of MSC-EXO [120]. In addition, current MSC-EXO isolation techniques are unable to fully separate EXO from impurities like cell debris and protein aggregates. These impurities reduce the concentration of the active components in EXO preparations and may introduce experimental variability, thereby interfering with therapeutic effect evaluation. MSC-EXO quality is affected by multiple variables: first, variations in MSC tissue sources directly affect the biological properties of derived EXO. However, no studies have yet compared the therapeutic effects of MSC-EXO from different tissue origins on POI under uniform experimental conditions. Therefore, the optimal tissue origin of MSC-EXO cannot be determined yet. Second, culture environment variables (e.g., nutrient composition, oxygen tension) can cause batch-to-batch quality variability. Third, EXO isolated using different techniques exhibit distinct particle size distributions and biological activities. Finally, storage conditions (e.g., temperature and duration) directly affect exosomal structural integrity and functional stability [121]. Therefore, further research is required to identify the optimal cell source. A standardized system for EXO extraction, identification, storage, and quality assessment still remains to be established.

Long-Term safety and efficacy issues

For long-term safety evaluation, while no significant toxic effects or histopathological changes have been detected in short-term MSC-EXO interventions in POI animal models, potential risks associated with prolonged exposure require carefully assessment. For instance, whether long-term administration could induce abnormal cell proliferation, differentiation dysregulation *via* disruption of cellular homeostasis, or even increase tumorigenic risk. These issues urgently require clarification through long-term follow-up investigations [122]. Regarding long-term efficacy, current animal studies are limited by short observation periods, leaving the long-term effects of MSC-EXO treatment unclear. Although experimental data demonstrated improved reproductive function markers following MSC-EXO treatment, whether ovarian function can be sustained at normal levels post-partum requires further confirmation [21, 98]. In addition, rodent POI models (mice/rats) typically have short pregnancy cycles (approximately 20 days), markedly distinct from the human physiological pregnancy duration of up to 40 weeks. This discrepancy undermines the temporal relevance of EXO treatment in humans - whether their biological activity can sustain throughout the entire human pregnancy cycle requires validation *via* translational research. Furthermore, interspecies biological differences in ovarian aging mechanisms and tissue regeneration capacity challenge the external validity of extrapolating animal study results to clinical applications.

Low homing efficiency of EXO

Currently, the primary delivery strategies for MSC-EXO in POI treatment include 3 administration methods: intravenous injection, intraperitoneal injection, and in-situ ovarian injection. Although intravenous injection offers benefits such as operational convenience, high safety, and cost-effectiveness, experimental evidence indicates markedly restricted targeted delivery efficiency [123]. Following intravenous injection, MSC-EXO undergo the following processes [124]: (1) circulation in the bloodstream, with most being cleared by the liver and spleen; (2) penetration of the vascular endothelial barrier and extracellular matrix; (3) uptake by target cells. Therefore, intravenous injection results in predominant accumulation of MSC-EXO in the liver, spleen, lungs, and kidneys, with minimal ovarian distribution [20, 125, 126]. Similar to most other nanoparticle - based drug carriers, sole reliance on the intrinsic properties of MSC-EXO falls short of achieving precise POI treatment and targeted delivery. Intraperitoneal injection suffers from the same limitation. Although this method elevates local drug concentration, it fails to enable precise delivery. Regarding in-situ ovarian injection, while a lower MSC-EXO dose suffices to achieve the same therapeutic efficacy, open-abdominal in-situ ovarian injection imposes significant physical damage, potentially leading to tissue trauma and postoperative complications. Ultrasound-guided minimally invasive injection mitigates these risks but necessitates specialized imaging equipment and trained operators. Moreover, since the ovary is a solid organ rather than a spongy structure like the joint cavity, it is not suitable for injecting a large amount of MSC-EXO. Forced injection inevitably risks local mechanical ovarian damage [127]. Furthermore, rodent ovarian models rely

on the cystic envelop surrounding the mouse/rat ovary, whereas the human ovary lacks this structure, rendering intracystic injection technically infeasible in clinical settings. Therefore, developing delivery technologies aligned with human anatomical characteristics and conducting rigorous preclinical safety evaluations represent critical research directions for optimizing the MSC-EXO delivery strategies.

Prospects of MSC-EXO in the treatment of POI

Optimizing the Production and Delivery Technologies of MSC-EXO

Developing novel production technologies is pivotal for enhancing the yield and quality of MSC-EXO. Although numerous separation and purification techniques have been developed, each exhibits limitations such as low specific capture efficiency or difficulties in large-scale manufacturing, thereby hindering standardized mass production of MSC-EXO. At present, researchers are actively exploring emerging technologies (e.g., ExoQuick, size exclusion chromatography, microfluidics) to overcome limitations of existing methods [42]. In addition, three-dimensional (3D) culture technology represents a critical avenue for optimizing MSC-EXO preparation [128–130]. The 3D culture technology provides MSCs with a microenvironment closer to the in vivo physiological state, facilitating cell growth, differentiation, and enhanced MSC-EXO secretion efficiency [131]. Compared to two-dimensional static culture system, 3D culture technology can increase MSC-EXO yield by an order of magnitude, with improved biological activity and function of the secreted EXO [131]. This offers a novel approach for the standardized production of clinical-grade MSC-EXO.

Targeted delivery technology of EXO holds critical importance for enhancing therapeutic efficacy and minimizing adverse effects. The excellent nanoscale properties of EXO render them ideal candidates for ovarian-targeted therapy. For example, EXO can traverse the blood-follicle barrier to exert therapeutic effects [69]; their surface-expressed CD47 reduces susceptibility to degradation, conferring a prolonged circulation half-life [132]; MSC-EXO exhibit intrinsic anti-inflammatory and regenerative properties [133]; their cargo–including nucleic acids, proteins, and encapsulated drugs–can be genetically or chemically modified, thereby significantly enhancing their therapeutic potential [134]. Therefore, addressing the challenge of suboptimal biodistribution and developing ovarian-targeted delivery strategies for EXO are critical. Effective targeted delivery of EXO requires membrane-targeting modification or magnetic targeting strategies. Membrane-targeting modification leverages the phospholipid bilayer membrane structure of EXO. Genetic engineering and chemical modification techniques enable manipulation of surface proteins to express or conjugate single-chain antibodies, targeted peptides, or nanobodies on their membrane surface.

Genetic engineering enables the modification of EXO to express specific targeting molecules. Bellavia et al. engineered a plasmid encoding recombinant human lysosomal-associated membrane protein 2b (Lamp2b) and interleukin-3 (IL-3) and generated IL3-Lamp2b-expressing EXO *via* HEK293T cell transfection. These HEK293T cells were subsequently transfected with imatinib/BCR-ABL siRNA at varying concentrations to produce drug- and siRNA-loaded EXO for cancer therapy. The results demonstrated that engineered EXO selectively targeted chronic myeloid leukemia cells, delivering anti-tumor drugs and potently inhibiting cancer cell growth in both in vitro and in vivo models, thus paving the way for overcoming drug resistance in chronic myeloid leukemia [135]. Alvarez-Erviti et al. introduced a neuron-specific rabies virus glycoprotein peptide—encoding vector into cells, enabling its fusion with Lamp2b and subsequent delivery of siRNA-loaded EXO to the brain for therapeutic action [136]. Similar examples of Lamp2b fusion expression of targeting peptides include targeting of cardiomyocytes [137, 138], breast cancer cells [139], and chondrocytes [140]. Zou et al. surface-expressed a high-affinity human immunodeficiency virus-1-specific single-chain antibody fragment on EXO, co-loaded them with curcumin and miR-143, and achieved selective targeting and killing virus-infected cells [141]. This highlights the potential to identify ovarian tissue-specific targeting peptides or antibody fragments and fuse them to EXO membrane proteins (e.g., Lamp2b) to enhance targeting and homing efficiency. Ovarian cell-targeting peptides can be fused to EXO membrane protein Lamp2b, and upon MSC transduction, the secreted EXO express this fusion protein on their surface, thereby enabling targeted delivery to ovarian cells. This method significantly enhances EXO accumulation in ovarian tissues, amplifies therapeutic efficacy, mitigates non-specific interactions with peripheral tissues, and reduces adverse effects.

Different from the genetic engineering method that rely on parental cells, chemical modification represents a direct intervention strategy for EXO, including bioconjugation, amidation, aldehyde-amine condensation, click chemistry, hydrophobic insertion, receptor-ligand binding, and multivalent electrostatic interactions, etc [142]. Cui et al. conjugated the bone-targeting peptide Ser-Asp-Ser-Ser-Asp to the membrane of MSC-EXO through diacyl lipid insertion and subsequently loaded siRNA into EXO by electroporation, enabling specific targeting and delivery of siRNA to osteoblasts and exhibiting significant therapeutic efficacy in osteoporosis treatment [143].

While various EXO modification strategies exist, the identification of ovarian-specific targets remains a

critical challenge. An ideal targeting molecule should satisfy 2 key criteria: first, it needs to be located in the cell membrane surface domain, such as a membrane receptor or ligand; second, it needs to have ovarian tissue specificity or be highly expressed in the ovary. Given the physiological characteristics of the ZP barrier and reproductive safety considerations, direct targeting of oocytes warrants careful evaluation. In contrast, granulosa cells—present in all follicular development stages, adjacent to oocytes, and central to POI pathogenesis—emerge as more promising targeting candidates. Current evidence suggests that the granulosa cell-specific marker, follicle stimulating hormone receptor (FSHR), represents a promising ovarian-specific target [127]. FSHR is continuously highly expressed on granulosa cell membranes throughout preantral follicle to mature follicle development, with undetectable or minimal expression in extra-ovarian tissues. This expression specificity provides a theoretical basis for it to be used as a target. However, the efficacy and long-term safety of FSHR-targeted ovarian delivery strategies remain to be elucidated. In addition to FSHR, single-cell sequencing and proteomics can systematically screen ovarian-specific membrane surface markers, enabling the discovery of novel target molecules and providing a basis for constructing intelligent targeted MSC-EXO delivery systems.

Targeted delivery can also be achieved by incorporating magnetic nanoparticles into EXO and applying an external magnetic field. Li et al. co-cultured MSCs with superparamagnetic iron oxide nanoparticles for 16 h to generate MSC-EXO loaded with magnetic nanoparticles. Under external magnetic field guidance, EXO accumulation at the skin injury site was significantly enhanced, leading to improved repair outcomes [144]. Kim et al. also achieved significant targeted therapeutic effects in ischemic stroke and spinal cord injury models using magnetic nanoparticle-loaded MSC-EXO guided by an external magnetic field [145, 146]. However, since the ovary is deeply located in the pelvic cavity and inaccessible to in vitro positioning, the applicability and optimal implementation of this method require further investigation.

Comprehensive Investigation into the Therapeutic Mechanism of MSC-EXO

Although many studies have explored the active components and mechanisms of MSC-EXO in treating POI, MSC-EXO from different tissue sources exhibit significant heterogeneity in their active ingredients and modes of action. Additionally, there is a lack of systematic analysis of the specific molecular targets, signaling pathways, and spatiotemporal effects of the bioactive components delivered by MSC-EXO during their in vivo actions, necessitating more in-depth mechanistic research. Currently, most findings are based on in vitro experiments or animal models, leaving substantial gaps in our understanding of the pharmacokinetics, tissue tropism, and long-term safety of MSC-EXO in humans. As a result, clinical applications lack clear theoretical foundations for efficacy evaluation and dose optimization. Therefore, elucidating the precise mechanism of action of MSC-EXO is a critical prerequisite for advancing their translation from basic research to clinical practice.

Systematic dissection of EXO therapeutic mechanisms necessitates an integrated multi-omics research strategy. Combined use of proteomics, transcriptomics, and metabolomics technologies advances MSC-EXO therapeutic mechanism research to the molecular network level. Proteomics systematically characterizes the protein cargo encapsulated within EXO, identifies key effector proteins, and elucidates their mechanistic roles in POI treatment [147]. By comparing protein expression profiles across physiological, POI pathological, and EXO-treated ovarian tissues, researchers can construct disease-specific protein regulatory networks and clarify spatiotemporal expression patterns of key proteins during POI pathogenesis and treatment. This dynamic analysis not only verifies the delivery of therapeutic proteins to damaged ovarian tissues but also reveals treatment-induced ovarian proteome remodeling features. Typical studies have demonstrated that hBMSC-EXO enhance ovarian function through the secretion of key protein YB-1 [82].

Transcriptomics enables characterization of the RNA expression profile within EXO, deciphering the functions of messenger RNA, miR, long non-coding RNA, circRNA carried by EXO in POI therapy [148]. Numerous studies have found that miRs and circRNAs in EXO regulate ovarian cell proliferation, apoptosis, and differentiation by modulating target gene expression, thereby improving ovarian function in POI animal models [62, 66, 67, 71]. Therefore, transcriptomics-based screening enables in-depth investigation of effective cargos, target genes and signaling pathways to provide a solid theoretical basis for therapeutic development. Moreover, metabolomics elucidates how EXO modulate ovarian cell metabolism, uncovering their mechanistic toles in regulating energy and lipid metabolism pathways in ovarian cells.

Moreover, single-cell sequencing is pivotal for mechanistic exploration. Single-cell sequencing enables high-throughput profiling of the genome, transcriptome, and epigenome at single-cell resolution, facilitating detailed elucidation of EXO effects on distinct ovarian cell types. Ovarian tissue comprises various cell types–ovarian granulosa cells, stromal cells, endothelial cells, monocytes, T cells—each contributing uniquely to ovarian homeostasis. Granulosa cells play an important role in POI pathogenesis, though not the sole contributors. Single-cell sequencing of rhesus monkey ovarian tissue

following hUCMSCs treatment identified upregulated anti-aging genes primarily expressed in granulosa cells, ovarian endothelial cells, and monocytes [149]. Therefore, the authors speculated that hUCMSCs play an anti-ovarian aging role mainly by actively regulating the functions and interactions of granulosa cells, endothelial cells, and monocytes, thus constructing a robust vascular niche, mitigating ovarian inflammatory response, and promoting oocyte maturation. Currently, many studies have carried out single-cell sequencing on tissues such as skin damage [150], spinal cord injury [151], and muscle degenerative diseases [152] after MSC-EXO treatment, attempting to clarify the therapeutic mechanism of MSC-EXO. However, no studies have reported the use of single-cell sequencing to analyze cellular changes in the ovarian microenvironment after MSC-EXO intervention. With the rapid development of science and technology, utilizing various sequencing technologies to uncover the active components and therapeutic mechanisms of MSC-EXO will rapidly advance their translational applications.

Conclusion

POI severely affects the physical and mental health as well as the fertility of women of childbearing age, as no effective therapeutic strategies have been established to date. As an emerging therapeutic approach, MSC-EXO offer promising potential for POI treatment. MSC-EXO carry a variety of bioactive molecules into ovarian cells, thereby regulating multiple cellular pathways to inhibit ovarian granulosa cell apoptosis, promote cell proliferation and differentiation, alleviate immune inflammatory responses and oxidative stress, inhibit fibrosis, and enhance ovarian angiogenesis—ultimately improving ovarian function. However, MSC-EXO-based therapy for POI encounters substantial challenges, including non-standardized preparation process, undefined long-term safety/efficacy profiles, lack of targeted delivery systems, etc.—all of which hinder its clinical translation and widespread implementation. Therefore, only by optimizing MSC-EXO preparation/delivery systems, deciphering their mechanistic basis, and achieving high-quality, controllable targeted repair of ovarian tissue damage, can we promote the clinical application and bring more hope and well-being to POI patients.

Abbreviations

POI	Premature ovarian insufficiency
FSH	Follicle stimulating hormone
MSCs	Mesenchymal stem cells
EXO	Exosomes
DNA	Deoxyribonucleic acid
miR	Micro ribonucleic acid
RNA	Ribonucleic acid
hUCMSCs-EXO	Human umbilical cord mesenchymal stem cell-derived exosomes
circRNAs	Circular RNAs
hBMSC-EXO	Human bone mesenchymal stem cell-derived exosomes
hAFMSC-EXO	Human amniotic fluid mesenchymal stem cell-derived exosomes
hAMSC-EXO	Human amniotic mesenchymal stem cell-derived exosomes
hADMSC-EXO	Human adipose mesenchymal stem cell-derived exosomes
hMenMSC-EXO	Human menstrual blood mesenchymal stem cell-derived exosomes
TSP1	Thrombospondin-1
VCD	4-vinylcyclohexene dicycloxide
CTX	Cyclophosphamide
ZP	Zona pellucida
TG	Tripterygium glycosides
IP	Intraperitoneal injection
ISI	In situ injection
HI	Hypodermic injection
UC	Umbilical cord
AT	Adipose tissue
BM	Bone marrow
AF	Amniotic fluid
MenB	Menstrual blood
3D	Three-dimensional
Lamp2b	Lysosomal-associated membrane protein 2b
IL-3	Interleukin-3
FSHR	Follicle stimulating hormone receptor

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

Xiao Zhang conceived the study, searched for relevant literatures, made the figures and drafted the manuscript. Shaowei Wang guided manuscript preparation and revised the manuscript. All authors read and approved the final manuscript.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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