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REVIEW

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Mesenchymal stem cell-derived extracellular vesicles therapy for primary ovarian insufficiency: a systematic review and meta-analysis of pre-clinical studies

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Abstract

Background Primary ovarian insufficiency (POI) manifests with hormonal imbalances, menstrual irregularities, follicle loss, and infertility. Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) are emerging as a promising treatment for POI. This systematic review aims to assess the effects of MSC-EVs on follicle number, hormonal profile, and fertility in POI animal models.

Methods A systematic search of PubMed, Scopus, and Web of Science databases up to December 14th, 2023 was conducted. Two reviewers independently conducted screening, risk of bias assessment, and data extraction. Meta-analysis was performed to analyze treatment versus control outcomes using a random effects model. Publication bias was assessed using Egger's regression test and sensitivity analysis was assessed using the leave-one-out method. Subgroup analyses and meta-regressions were conducted based on EV source, induction model, type of animal, study quality, administration route, administration frequency and route, and dose.

Results a total of 29 studies were included. MSC-EVs treatment significantly increased total follicle count (SMD, (95CI), p-value; 3.56, (0.91, 6.21), < 0.001), including primordial (SMD, (95CI), p-value; 2.86, (1.60, 4.12), < 0.001), primary (SMD, (95CI), p-value; 3.17, (2.28, 4.06), < 0.001), mature (SMD, (95CI), p-value; 2.26, (1.02, 3.50), < 0.001), and antral follicles (SMD, (95CI), p-value; 2.44, (1.21, 3.67), < 0.001). Administration frequency and route did not affect this outcome, but EV source affected primordial, primary, secondary and antral follicle count. Additionally, MSC-EVs treatment elevated anti-müllerian hormone (SMD, (95CI); 3.36, (2.14, 4.58)) and estradiol (SMD, (95CI); 3.19, (2.20, 4.17)) levels while reducing follicle stimulating hormone levels (SMD, (95CI); -2.68, (-4.42, -0.94)). Unlike EV source, which had a significant impact on all three hormones, administration frequency, route, and EV dose did not affect this outcome. Moreover, treatment increased offspring number (SMD, (95CI); 3.70, (2.17, 5.23)) and pregnancy odds (OR, (95CI); 10.25, (4.29,

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24.46)) compared to controls. Publication bias and a high level of heterogeneity was evident in all analyses, except for the analysis of the pregnancy odds. However, sensitivity analysis indicated that all of the analyses were stable.

Conclusion MSC-EVs therapy shows promise for POI treatment, potentially facilitating clinical translation. However, Further research is warranted to optimize methodology and assess side effects.

Keywords Extracellular vesicle, Mesenchymal stem-cell, Primary ovarian insufficiency, Animal studies, Exosome, Preclinical

Introduction

Primary ovarian insufficiency (POI) is the deterioration of ovarian function, diminished follicle reserves, and increased follicle stimulating hormone (FSH) levels before the age of 40, which results in diminished production of estrogen and progesterone and lower health related quality of life [1–3]. With a global prevalence of 3.7%, it is commonly caused by genetic mutations in apoptotic, cell cycle, and folliculogenesis pathways [1], such as the PI3k/AKT/mTOR pathway [4], yet it can be caused by radiotherapy or chemotherapy as well. The diminished follicle reserves seen in POI results in reduced serum anti-müllerian hormone (AMH) levels [5] and infertility, while the lowered estrogen levels are the likely culprit behind the higher risk of developing ischemic heart disease [6] and dementia [7].

Current treatments like hormone replacement therapy present challenges due to high costs, limited availability, and potential side effects [8]. In the case of premenopausal women, oral contraceptives (OCP) may be an alternative treatment, which increase the risk of venous thromboembolism and insufficient daily physiologic repletion [9] and pose a challenge for women who harbor fertility aspirations [10]. This highlights the need for novel therapies that preserve fertility and increase estrogen levels, such as mesenchymal stem cells (MSCs) and their derived extracellular vesicles (MSC-EVs).

MSCs have been used extensively in clinical studies, serving as a regenerative agent for a spectrum of conditions, including osteoarthritis, pulmonary fibrosis, spinal cord injury, and myocardial damage [11–13]. Their application for treating POI has been tested in a plethora of POI animal models, with a recent meta-analysis finding they increased AMH levels and follicle counts alongside elevating estrogen levels and fertility in POI modelled animals [14]. This therapeutic effect is believed to be caused by paracrine secretions, most notably extracellular vesicles.

Extracellular vesicles (EVs) are small structures that transport lipids, miRNAs, and proteins to other cells, influencing a broad spectrum of biological processes such as gene expression and cell function [15, 16]. MSC-EVs in particular are capable of modulating the immune system, inhibiting apoptosis, regenerating soft tissue, and regulating inflammation [17–19]. Due to their innate capacity to modulate inflammation, MSC-EVs hold significant therapeutic potential and have already shown promise in treating POI in animal models [20, 21]. Their characteristics, including biocompatibility, low toxicity, minimal immunogenicity, and membrane permeability, position them as an ideal carrier for delivering diverse drugs for targeting of inflammatory processes [22, 23]. As such, EVs may be a potential alternative therapeutic approach in POI [24].

With this in mind, the effectiveness of MSC-EVs for treating POI has yet to be systematically reviewed and assessed, therefore, we aim to systematically assess and meta-analyze the effectiveness of MSC-EVs on fertility parameters of POI in animal models. These models are essential for evaluating the safety and efficacy of potential treatments, ultimately guiding the development of future clinical trials and improving patient care. Chief among these models for POI is the chemotherapy model, which has been observed to reduce follicle count and impair fertility, yet other models such as galactose induced or 4-vinylcyclohexene diepoxide induced models have also shown similar declines in fertility and follicle count [25]. To assess the effectiveness of MSC-EVs for treating POI, we compared fertility parameters (follicle counts, serum hormone levels (FSH, AMH, estradiol, luteinizing hormone), and fertility through the mean number of offspring and number of pregnant animals) in the MSC-EVs treated animals to untreated animals.

Methods

This systematic review and meta-analysis adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [26]. The protocol of this review was prospectively registered on the International Prospective Register of Systematic Reviews (PROSPERO) with the registration ID: CRD**42024519963**.

Search Strategy

The literature search was conducted using PubMed, Scopus, and Web of Science databases on December 14th, 2023 with keywords synonymous with ("mesenchymal stem cells" OR "mesenchymal stromal cells"), AND ("extracellular vesicles" OR "exosome"), AND ("primary ovarian insufficiency" OR "POI"), AND ("fertility" OR

"follicle" OR "estradiol" OR "anti-Müllerian hormone" OR "luteinizing hormone", OR "follicle-stimulating hormone"). We did not impose any limitations on language or publication year.

Eligibility criteria

Studies were included if they complied with our predefined PICOS criteria (Table 1). Studies were excluded if they used none mesenchymal stem cells or if they were exclusively in vitro or ex vivo studies. Synthetic nanoparticles were excluded as they do not meet the International Society of Extracellular Vesicles' definition of an extracellular vesicle [27, 28].

Study selection

After the removal of duplicate records, the process of study screening and selection was undertaken by two independent reviewers (I.M., K.G.) each screening the studies in two phases. First, the articles were screened using their title/abstracts, with the selected articles being sought for full-text retrieval. During the second phase, the full text of the articles was examined to include studies that complied with the predefined PICOS criteria. Discrepancies between the two reviewers were settled by a third reviewer through discussion (S.R.F.).

Data extraction

The collection of relevant data was independently performed by two reviewers (S.R.F., S.A.M.), who extracted the following data into a pre-designed Excel spreadsheet: first author, year of publication, animal species, POI model, sample size, source of EV, isolation method, EV validation, administration route, cargo and pathway implicated in the therapeutic effects of EVs, time from treatment to sacrifice, follicle type and count, serum hormone type and level, pregnancy rate, and number of offspring. Much of the data was reported using graphical representations across the included studies; therefore, we used PlotDigitizer [29] to accurately extract means and standard deviations from the graphs. Discrepancies were resolved by an independent reviewer through discussion (I.M.).

Isolation methods utilized in the included studies were categorized and recorded based on the definitions provided by Chen et al. [30]. Additionally, the particular characteristics of extracellular vesicles required by the International Society of Extracellular Vesicles were extracted under the EV validation heading in the datasheet [28]. Separation of human and animal derived mesenchymal stem cells by their source, i.e. bone marrow, was based on research highlighting their significant therapeutic differences [31, 32].

Outcomes

The following were chosen as the primary endpoints for fertility assessed by this review: follicle count, pregnancy rate, number of offspring, and serum levels of hormones related to ovarian function namely estradiol (E2), anti-Müllerian hormone (AMH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH).

Quality assessment

The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool [33] was used to measure the risk of bias for each of the included studies by two independent reviewers (K.G., I.M.). This tool assesses six types of bias, namely selection bias, performance bias, detection bias, attrition bias, reporting bias, and other sources of bias. Table S1 delineates the questions that assessed each domain of bias. The included studies were judged to have a low, unclear, or high risk of each type of bias mentioned. Discrepancies were resolved through discussion with a third reviewer (S.R.F.), and a graphical representation of the data was created using the robvis shiny web app [34]. A score of >7 was considered high quality. When at least 10 studies were included in a meta-analysis including low quality studies, a sensitivity analysis comparing low quality studies with moderate/high quality ones was conducted.

Statistical analysis

Statistical analysis was planned for follicle count, pregnancy rate, number of offspring, and serum hormone levels, and was conducted using Stata version 17 (StataCorp), with means and standard deviations being the only acceptable data entry form for the follicle count, number of offspring, and serum hormone levels meta-analyses. The number of pregnant animals in the untreated POI and EV-treated arms was the only acceptable data entry form for the pregnancy rate meta-analysis. A

Table 1 The predefined eligibility criteria based on population, intervention, comparator, outcome, and study design (PICOS)

Population	Animal models of POI. No weight or animal species limitations were defined.
Intervention	Extracellular vesicles derived from mesenchymal stem cells. No administration route, dose, timing, or particle size limitations were defined.
Comparator	Animal models of primary ovarian insufficiency that have not received any treatment, or have been administered a placebo.
Outcome	Fertility parameters (Follicle count, pregnancy rate, number of offspring, and serum levels of hormones related to ovarian function namely estradiol (E2), anti-Müllerian hormone (AMH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH)).
Study design	Controlled studies with at least two separate untreated POI and EV-treated POI arms.

random-effects model was utilized due to a high amount of heterogeneity in the treatment regimens and POI models used in the included studies. We used standardized mean difference (SMD), a 95% confidence interval (CI), and a two-tailed p-value to report the pooled effect size for the follicle count, number of offspring, and serum hormone levels meta-analyses. The pooled effect size for the pregnancy rate meta-analysis was reported using Odds Ratio (OR) and a 95% confidence interval. Statistical significance was set at <0.05. Heterogeneity was assessed using the I_2 statistic, with an I_2 of $>50\%$ signifying high heterogeneity [35]. Meta-analysis was conducted if at least 5 studies reported their numerical findings. If at least 10 studies were included in the meta-analysis, a subgroup analysis based on EV source, induction model, type of animal, study quality, and administration route was conducted. Meta-regression of administration frequency and total administered dose was also conducted if at least 10 of the studies in the meta-analysis had reported their numbers.

Sensitivity analysis was conducted using the leave-one-out method to ensure the robustness of our findings. Publication bias was evaluated by Egger's regression test alongside funnel plot symmetry.

Results

Our online search yielded a total of 279 articles, of which 190 were chosen for title-abstract screening after duplicate removal. 36 of these 190 papers were chosen for full-text evaluation and 29 were included in our review (Fig. 1). The reason for the exclusion of papers in the full text evaluation phase can be found in Table S2.

The 29 included studies mostly studied MSC-EVs' therapeutic effects on chemotherapy-induced POI models with the exception of Song et al. [21], Zhang et al. [36], and Ding and Qian et al. [37] who used a 4-vinyl cyclohexene diepoxide induced model, Yang et al. [38] who used a natural aging model, and Li and Fan et al. [39] who used a D-galactose-induced aging model. The animals used in the models were mostly C57BL/6J mice with the exception of 15 studies [21, 36, 38, 40–51]. All studies that mentioned their housing conditions kept the animals in 10–14-hour light-dark cycles and 25–27-degree temperatures.

Regarding the extraction of extracellular vesicles, a wide host of mesenchymal stem cells were utilized as a source, including human umbilical cord mesenchymal stem cells (H-UCMSC) used in 14 studies [20, 38, 39, 41, 43–45, 50–56], human amniotic fluid mesenchymal stem cells (H-AFMSC) used in 4 studies [37, 40, 57, 58], induced pluripotent mesenchymal stem cells (iPSC-MSCs) used in two studies [59, 60], human bone marrow mesenchymal stem cells (H-BMSC) used in two studies [54, 61], human menstrual blood mesenchymal stem cells used in two studies [21, 36], murine amniotic fluid mesenchymal stem cells (M-AFMSC) used in two studies [42, 46], murine bone marrow mesenchymal stem cells (M-BMSC) used in two studies [47, 48], human clonal mesenchymal stem cells (H-cMSC) used in one study [62], and ewe amniotic fluid mesenchymal stem cells (E-AFMSC) used in one study [49].

The treatment regimen was very heterogeneous, with doses per administration ranging from 10 µg [38, 49] to 400 µg [50], although the administration frequency was usually once, with the exception of 11 studies [21, 36, 39, 43, 44, 47, 48, 51, 56–59, 61]. The total dose of extracellular vesicles administered was not calculable for most of the studies due to not reporting the protein content of their extracellular vesicles, yet for those that quantified the protein content a total dose of 10 [38, 49] to 1200 [59] µg was administered with most giving between 100 and 250 µg of extracellular vesicles in total. The administration route was mostly intravenous, with the exception of 11 studies using an intra-ovarian route and 3 using an intraperitoneal route.

16 of the included studies analyzed the contents of their extracellular vesicles and implicated numerous miRNAs and one protein in the therapeutic response they observed. These included miR-21-5p and miR-22-3p which were implicated in two studies each [20, 38, 59], signifying a stronger likelihood of their implication. 21 of the included studies investigated the molecular pathways these extracellular vesicles utilized to exert their therapeutic response and 9 of them found the activation of the PI3K/AKT pathway was responsible [37–39, 44, 48, 50, 58, 59, 62], yet the activation of the Wnt/β-Catenin pathway [20], the SMAD pathway [21, 40, 49], and the Hippo pathway [56] have also been observed after MSC-EVs treatment and have been attributed to its therapeutic response (Table 2).

The effectiveness of MSC-EVs for the treatment of infertility in POI was investigated by studying the following fertility parameters:

Follicle Count

The mean number of follicles after treatment was assessed by 20 studies [21, 36, 40–44, 46, 47, 49, 51–53, 55–60, 62]. For counting follicles, serial sections between 3 and 8 µm were taken from the ovaries, with 4 studies [21, 59, 60, 62] taking one of every 5 serially sectioned ovary samples and multiplying their count by 5, two studies [36, 43] took one of every 5 serial sections and summed their counts, two studies [44, 52] took one of every 10 sections and averaged the number of each follicle type counted, two studies [42, 46] took one of every 10 serial sections and summed the number of each follicle type counted, one study [37] took 5 random sections from each ovary and counted the follicles, one study [60]

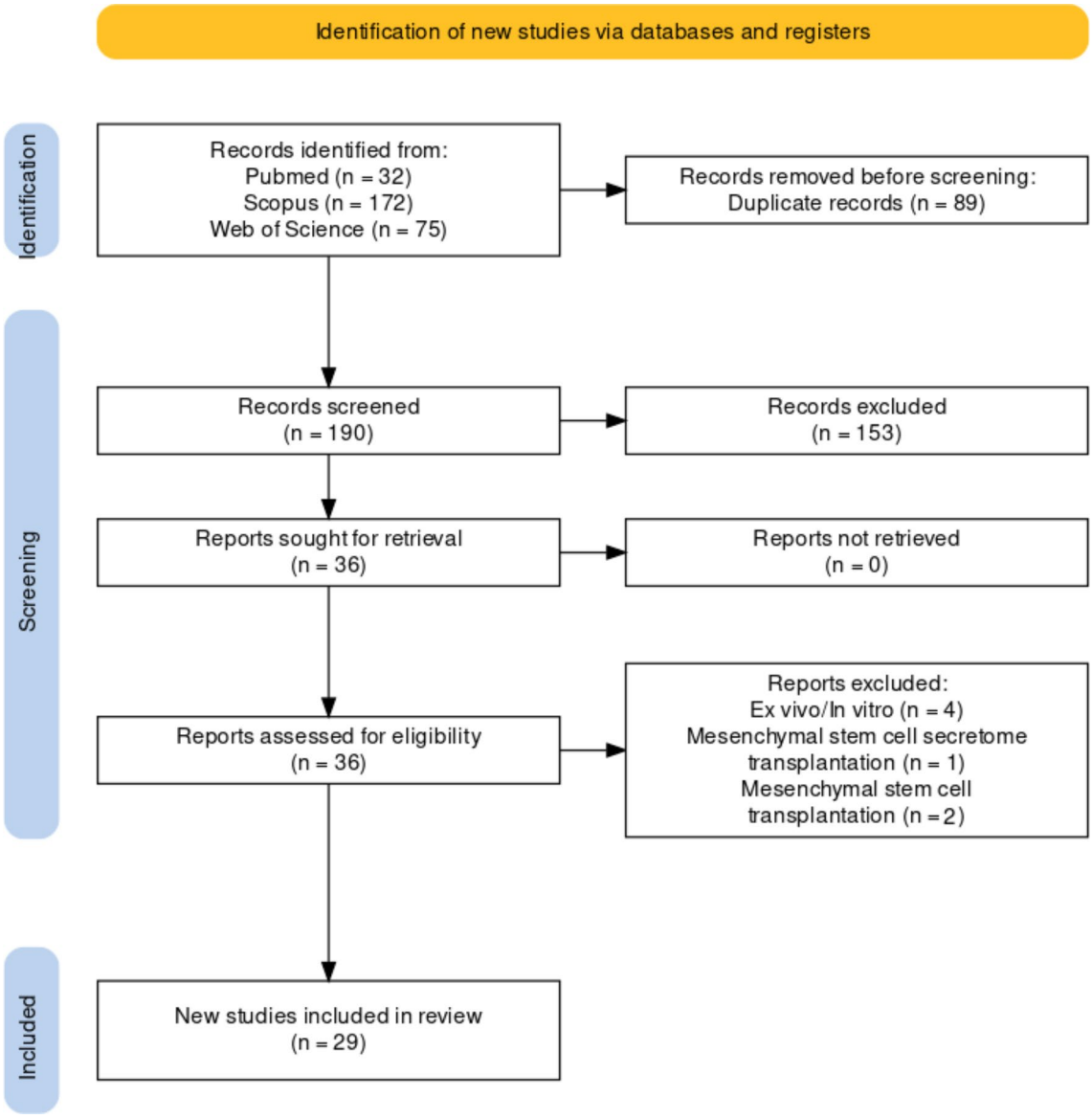

Fig. 1 PRISMA flowchart made using the tool by Haddaway et al

counted every follicle type in every serial section, one study [58] took one of every 5 serial sections and averaged the follicle counts, one study [56] took at least 3 random sections from serial sections and summed the count, and one study [40] took 3 representative samples three times from serial sections yet did not specify their summation process. 4 studies [41, 49, 51, 57] did not specify their methods.

The pooled results showed an increase in primordial follicles (SMD, (95CI), p-value; 2.86, (1.60, 4.12), <0.001) with high heterogeneity (I^2=89.95%) when comparing 104 MSC-EVs treated animals to 104 untreated ones, an increase in primary follicles (SMD, (95CI), p-value; 3.17, (2.28, 4.06), <0.001) with high heterogeneity (I^2=76.06%) when comparing 93 MSC-EVs treated animal to 93 untreated ones, an increase in secondary follicles (SMD, (95CI), p-value; 2.63, (1.47, 3.79), <0.001) with high heterogeneity (I^2=88.37%) when comparing 93 MSC-EVs treated animals to 93 untreated ones, an increase in mature follicles (SMD, (95CI), p-value; 2.26, (1.02, 3.50), <0.001) with high heterogeneity (I^2=82.28%) when comparing 48 MSC-EVs treated animals to 48 untreated ones, and an increase in antral follicles (SMD, (95CI), p-value; 2.44, (1.21, 3.67), <0.001) with high heterogeneity (I^2=87.20%) when comparing 73 MSC-EVs treated animals to 73 untreated ones. The total amount of follicles was also investigated in 7 studies and our meta-analysis showed a significant increase in their number (SMD, (95CI), p-value; 3.56, (0.91, 6.21), <0.001) with high heterogeneity (I^2=94.48%) when comparing 44 MSC-EVs

Table 2 Characteristics of the included studies

Author, Year	Number of Animals and Species	POI Model	Source of EV	Isolation Method	EV Validation	Treatment Model, Administration Route	Cargo implicated, Pathway Implicated	Time from Treatment to Sacrifice
Cao et al., 2023 [59]	36 C57BL/6J 28 days old	Chemotherapy induced, injected at day 5 and 11	iPSC-MSCs	Ultracentrifugation Ultrafiltration	TEM/flow nanoanalyzer Western blot for: CD9, CD63, CD81, HSP 70, Calnexin	EV dose: 200 µg Administration Frequency: 6 Regimen: treated on day 0, 2, 7, 9, 13, and 15 , Intravenous	miR-26a-5p, miR-22-3p, miR-221-3p, miR-21-5p, miR-92a-3p, miR-23a-3p, miR-181b-5p, miR-222-3p, miR-181a-5p, ILK-PI3K/AKT activation	48 h
Ding and Zhu et al., 2020 [55]	40 C57B6L/J 10-week-old	Chemotherapy induced	H-UCMSC	Exosome Separation Kit Differentiation Centrifugation	TEM/NTA Western blot for: CD9, CD63 and CD81	EV dose: 10^12 particles/mL Administration Frequency: 1 Regimen: treated once , intra-ovarian	miR-17-5p, SIRT7 gene inhibition	4 weeks
Eslami et al., 2023 [62]	44 C57BL/6 6–8 weeks old	Chemotherapy induced, injected 15 days consecutively	H-cMSC	Ultracentrifugation	SEM/DLS Western blot for: CD9, CD63, TSG101, Calnexin	EV dose: NP Administration Frequency: 1 Regimen: treated once , Intravenous	NA, PI3K/AKT activation	4 weeks
Gao et al., 2022 [52]	32 C57BL/6 8 weeks old	Chemotherapy induced, injected at day 0	H-UCMSC	Ultracentrifugation Ultrafiltration	Western blot for: CD63, Calnexin	EV dose: 125 µg Administration Frequency: 1 Regimen: treated on day 0 , Intravenous	miR-29a, Wnt/β-Catenin activation by inhibition of HBP1	15 days
Gao et al., 2023 [20]	40 C57BL/6 8 weeks old	Chemotherapy induced, injected 14 days consecutively	H-UCMSC	Exosome Separation Kit and ultrafiltration	TEM/NTA Western blot for: HSP70, TSG101, CD63, Calnexin	EV dose: 125 µg Administration Frequency: 1 Regimen: treated on days 15, 19, 24 , Intravenous	miR-22-3p, KLF6 and ATF4-ATF3-CHOP	5 days
Geng et al., 2022 [57]	36 C57B6L/J 10 week old	Chemotherapy induced, injected daily for a week and every other day for 2 weeks	H-AFMSC	Ultracentrifugation	TEM/ nanoFCM Western blot for: CD63, CD9, Calnexin	EV dose: 10^6 particles Administration Frequency: 14 Regimen: treated every other day for 4 weeks , Intravenous	miR-369-3p, YAF2 inhibition	NP
Huang et al., 2018 [40]	40 ICR 7-8 weeks old	Chemotherapy induced, injected 14 days consecutively	H-AFMSC	Exosome Separation Kit and ultrafiltration	TEM/Fluorescence-activated cell sorting (FACS) Flow cytometry for: CD63, CD9, and CD81	EV dose: produced by 10^6 cells Administration Frequency: 1 Regimen: treated once , intra-ovarian	NA, SMAD pathway activation	4 weeks

Table 2 (continued)

Author, Year	Number of Animals and Species	POI Model	Source of EV	Isolation Method	EV Validation	Treatment Model, Administration Route	Cargo implicated, Pathway Implicated	Time from Treatment to Sacrifice
Li and Fan et al., 2023 [39]	24 C57BL/6 6–8 weeks old	D-Galactose-induced aging, injected 42 days consecutevily	H-UCMSC	Ultracentrifugation	TEM/NTA Western blot for: CD9, CD63 and CD81, Calnexin	EV dose: 2*10^7/ml Administration Frequency: 21 Regimen: treated every other day , Intravenous	NA, PI3K/AKT activation	1 week
Li and Zhang et al., 2023 [53]	30 C57BL/6 6 week old	Chemotherapy induced, injected 15 days consecutively	H-UCMSC	Ultracentrifugation	TEM/NTA Western blot for: TSG101, CD9, and CD63	EV dose: prodcued by 2*10^6 cells Administration Frequency: 1 Regimen: treated once , intra-ovarian	NA, NA	4 weeks
Liu and Yin et al., 2020 [41]	174 ICR 5-6 weeks old	Chemotherapy induced, injected at day 0	H-UCMSC	Ultracentrifugation	TEM Western blot for: TSG101, CD9, and CD63, Calnexin	EV dose: 150 µg Administration Frequency: 1 Regimen: treated once 2 weeks after chemotherapy , Intravenous	NA, NA	4 weeks
Lu et al. 2023 [51]	36 Swiss albino rats 8 weeks old	Chemotherapy induced, injected 15 days consecutevily	H-UCMSC	Ultracentrifugation	TEM/Flow nano-analyzer Western blot for: CD63, CD81, TSG101, and Calnexin	EV dose: 50 µg Administration Frequency: 5 Regimen: treated once every 5 days , Intraperitoneal	miR-145–5p, Inhibition of XBP1	4 weeks
Nazdikbin Yamchi et al., 2023 [49]	21 Wistar rats 7-8 weeks old	Chemotherapy induced, injected 14 days consecutevily	E-AFMSC	Ultracentrifugation	SEM and TEM/DLS Western blot for: CD9, CD81, and CD63	EV dose: 10 µg Administration Frequency: 1 Regimen: treated once 3 weeks after chemotherapy , intra-ovarian	NA, Upregulation of Smad-4 and Smad-6	4 weeks
Park et al., 2023 [54]	C57BL/6	Chemotherapy induced, injected 7 days consecutevily	H-BMSC and H-UCMSC	NP	NP	EV dose: 1.5×10^7 Administration Frequency: 1 Regimen: treated once 7 days after chemtherapy induction , Intravenous	NA, NA	2 weeks

Table 2 (continued)

Author, Year	Number of Animals and Species	POI Model	Source of EV	Isolation Method	EV Validation	Treatment Model, Administration Route	Cargo implicated, Pathway Implicated	Time from Treatment to Sacrifice
Pu et al., 2023 [45]	36 Sprague Dawley rats 8 weeks old	Chemotherapy induced, injected 7 days consecutevily	H-UCMSC	NP	TEM/NTA Western blot for: CD9, CD63, and CD81	EV dose: 10^9 particles Administration Frequency: 1 Regimen: treated once 24 h after chemotherapy , Intravenous	NA, NA	4 weeks
Qu et al., 2022 [50]	105 Wistar rats 5 weeks old	Chemotherapy induced, injected 14 days consecutevily	H-UCMSC	Ultracentrifugation	TEM Western blot for: TSG101, Hsp70, CD63, and calnexin	EV dose: 400 µg Administration Frequency: 1 Regimen: treated once , Intravenous	miR-126-3p, PIK3R2 gene inhibition and subsequent PI3K/AKT/mTOR activation	4 weeks
Song et al., 2023 [21]	36 Sprague Dawley rats 6 weeks old	4-VD injection, injected 15 days consecutevily	menstrual blood	Ultracentrifugation and ultrafiltration	Western blot for: TSG101, CD81	EV dose: 4.5*10^7 Administration Frequency: 6 Regimen: treated every 5 days , Intravenous	thrombospondin-1, SMAD3/AKT/MDM2 activation and inhibition of P53	
Sun et al., 2019 [61]	15 C57BL/6 6 – 7 weeks old	Chemotherapy induced, injected once	H-BMSC	Exosome Seperation Kit and Centrifugation	TEM Western blot for: CD63, and calnexin	EV dose: 125 µg Administration Frequency: 3 Regimen: treated 1, 5, and 10 days after chemotherapy , Intravenous	miR-644-5p, inhibition of p53	5 days
Thabet et al., 2020 [46]	106 Sprague Dawley rats 6 weeks old	Chemotherapy induced, injected once	M-AFMSC	Ultracentrifugation and ultrafiltration	TEM/NTA	EV dose: 200 µg Administration Frequency: 1 Regimen: treated once 21 days after chemotherapy , intra-ovarian	miR-21, PTEN inhibition	1 day, 7 days, and 2 months
Xiao et al., 2016 [42]	ICR 4-6 weeks old	Chemotherapy induced, injected once	M-AFMSC	Exosome Seperation Kit and ultrafiltration	TEM/NTA	EV dose: 250 µg Administration Frequency: 1 Regimen: treated once 24 h after chemotherapy , intra-ovarian	miR-10a, inhibition of Bim which is downstream of p53 and upstream of Casp9	8 days

Table 2 (continued)

Author, Year	Number of Animals and Species	POI Model	Source of EV	Isolation Method	EV Validation	Treatment Model, Administration Route	Cargo implicated, Pathway Implicated	Time from Treatment to Sacrifice
Xiao et al., 2023 [43]	ICR 3 weeks old	Chemotherapy induced, injected 10 days consecutevily	H-UCMSC	Ultracentrifugation	TEM/NTA Western blot for: Alix, TSG101, and CD9	EV dose: NP Administration Frequency: 14 Regimen: treated daily for two weeks , Intravenous	NA, NA	2 weeks
Xing et al., 2023 [47]	Sprague Dawley rats 5 weeks old	Chemotherapy induced, injected 14 days consecutevily	M-BMSC	Exosome Seperation Kit and ultrafiltration	TEM/NTA Western blot for: CD9 and CD63	EV dose: 150 µg Administration Frequency: 7 Regimen: treated once every two days , Intravenous	miR-125a-3p, circLRRC8A activation and subsequent downregulation of NFE2L1	24 h
Yang and Lin et al., 2020 [48]	50 Sprague Dawley rats 5 weeks old	Chemotherapy induced, injected 14 days consecutevily	M-BMSC	Ultracentrifugation	TEM Western blot for: CD9, CD81, and tubulin	EV dose: 150 µg Administration Frequency: 7 Regimen: treated once every two days for 2 weeks , Intraperitoneal	miR-144-5p, activation of PI3K/AKT and subsequent inhibition of PTEN	4 weeks
Yang and Zhang et al., 2020 [38]	ICR 10 months old	Natural aging model	H-UCMSC	Ultracentrifugation	TEM/NTA Western blot for: Alix, TSG101, and CD9	EV dose: 10 µg Administration Frequency: 1 Regimen: NP , Intra-ovarian	miR-146a-5p or miR-21-5p, activation of PI3K/mTOR	3 weeks
Yang et al., 2019 [44]	60 ICR 4-5 weeks old	Chemotherapy induced, injected on day 0	H-UCMSC	NP	TEM Western blot for: CD9, CD63, TSG101, and Calnexin	EV dose: 150 µg Administration Frequency: 4 Regimen: treated once a week for 4 weeks , Intravenous	NA, activation of PI3K/AKT	4 weeks
Zhang et al., 2023 [60]	24 C57BL/6 8 weeks old	Chemotherapy induced, injected 14 days consecutevily	iPSC-MSCs	Ultracentrifugation	TEM/NTA Flow cytometry for: CD63 and CD9 Western blot for: Calnexin, TSG-101, and folittilin-1	EV dose: 25 µg Administration Frequency: 1 Regimen: treated once on the fifth day of modelling , Intra-ovarian	NA, NA	2 weeks

Table 2 (continued)

Author, Year	Number of Animals and Species	POI Model	Source of EV	Isolation Method	EV Validation	Treatment Model, Administration Route	Cargo implicated, Pathway Implicated	Time from Treatment to Sacrifice
Zhang et al., 2019 [58]	48 C57BL/6 7 – 8 weeks old	Chemotherapy induced, injected on day 0	H-AFMSC	Exosome Seperation Kit and ultrafiltration	TEM/NTA Western blot for: Alix, CD63, and CD9	EV dose: NP Administration Frequency: 2 Regimen: treated once a week for 2 weeks Intra-ovarian and , Intravenous	hsa-miR-1246, activation of PI3K pathway	4 weeks
Zhang et al., 2021 [36]	48 Sprague Dawley rats 7 weeks old	4-VD injection, injected 15 days consecutevily	menstrual blood	Ultracentrifugation and ultrafiltration	TEM/NTA Western blot for: TSG101 and CD81	EV dose: 50 µg Administration Frequency: 5 Regimen: treated once every five days , Intra-ovarian	NA, NA	4 weeks
Ding and Qian et al., 2020 [37]	40 C57BL/6 10 weeks old	4-VD injection, injected 14 days consecutevily	H-AFMSC	Exosome Seperation Kit and ultrafiltration	TEM Western blot for: Alix, TSG101, CD9, CD63, and CD81	EV dose: 150 µg Administration Frequency: 1 Regimen: treated once after model induction , Intra-ovarian	miR-320a, activation of PI3K/AKT pathway	4 weeks
Li et al., 2021 [56]	36 C57BL/6 8 weeks old	Chemotherapy induced, injected on day 0 and 7	H-UCMSC	Ultracentrifugation	TEM/NTA Flow cytometry for: CD9, CD63, and CD81	EV dose: 150 µg Administration Frequency: 2 Regimen: treated once every week , intraperitoneal	NA, Activation of the Hippo pathway	2 weeks

Abbreviations 4-VD: 4-vinylcyclohexene diepoxide; TEM: transmission electron microscopy; NTA: nano-particle tracking analysis; SEM: scanning electron microscopy; DLS: dynamic light scattering; NP: not provided; NA: not applicable

treated animals to 44 untreated ones (Fig. 2). No significant difference in the mean improvement of different follicle types is evident (p-value=0.83). Publication bias assessed using Egger's regression test showed significant publication bias for all the above analyses, while sensitivity analysis using the leave-one-out method showed stable results (Supplementary material 1, Figure S1-S12).

Follicle count and administration route

Subgroup analysis based on administration route was conducted for all above analyses except mature follicle count and total follicle count due to having less than 10 studies in their meta-analysis. no difference between intravenous, intra-ovarian, and intraperitoneal administration was observed in the antral follicle, primary follicle, primordial follicle, and secondary follicle meta-analysis (data not shown).

Follicle count and administration frequency

Meta-regression based on administration frequency for each follicle count showed no difference in the antral follicle meta-analysis (coefficient=-0.1533189, R2=0, p-value of explained variance=0.321), primary follicle meta-analysis (coefficient=-0.0303341, R2=0, p-value of explained variance=0.818), primordial follicle meta-analysis (coefficient=-0.0495191, R2=0, p-value of explained variance=0.791), and secondary follicle count (coefficient=0.789761, R2=0, p-value of explained variance=0.646). Total follicle count and mature follicle

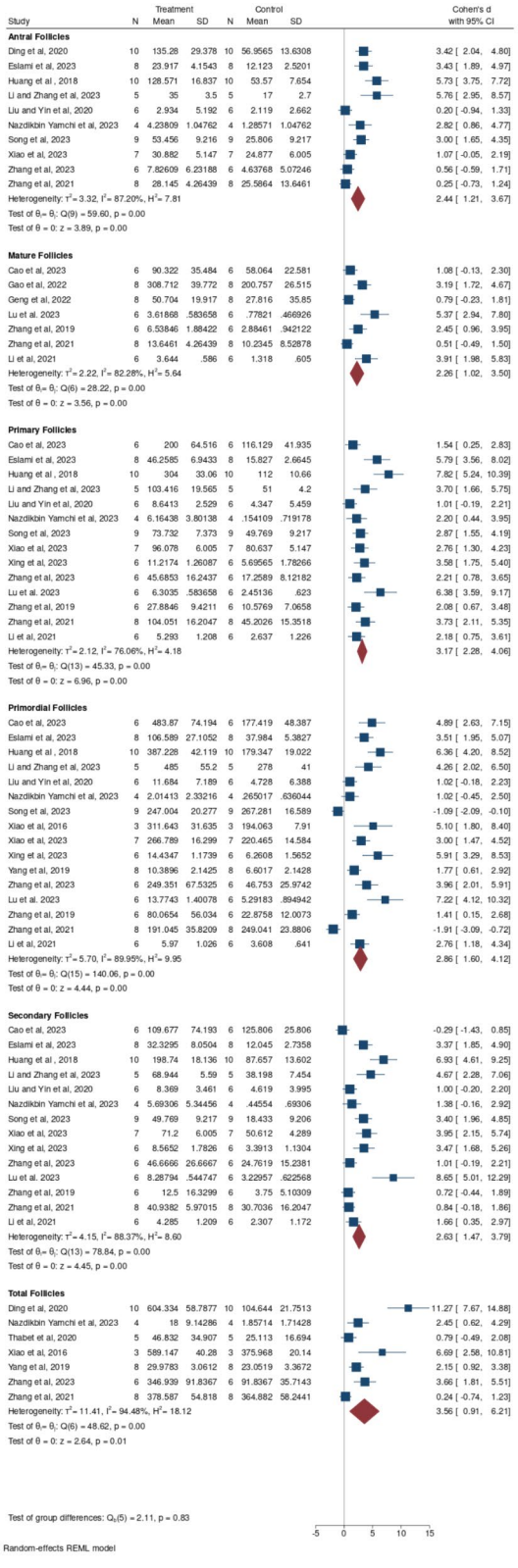

Fig. 2 Meta-analysis of Follicle counts after MSC-EV treatment subgrouped by the type of follicle

count meta-analyses could not be performed due to having less than 10 studies.

Follicle count and dose

Meta-regression based on dose showed no effect on the primordial follicle count (coefficient=0.0017907, R2=0, p-value of explained variance=0.371), while other follicle counts could not be analyzed due lack of reporting of MSC-EVs protein content in most studies.

Follicle count and EV source

Subgroup analysis based on EV source showed significant difference in primordial follicle count (p-value<0.001) with menstrual blood performing the poorest (SMD, (95CI), p-value; 2.86, (-2.22,-0.65), <0.001), a difference in primary follicle count (p-value=0.04) with H-cMSC performing significantly better than the rest (SMD, (95CI), p-value; 5.79, (3.56,8.02), <0.001), a difference in secondary follicle count (p-value=0.02), and a difference in antral follicle count (p-value<0.001) with H-AFMSC performing better than the rest (SMD, (95CI), p-value; 5.73, (3.75,7.72), <0.001). Mature and total follicle count meta-analyses were too few to be assessed.

Follicle count and induction model

Subgroup analysis based on induction model showed chemotherapy models performing better than 4-vinyl cyclohexene diepoxide models in the primordial follicle count (SMD (95CI): 3.44 (2.42,4.46) vs. -1.43 (-2.22, -0.65), p-value of subgroup difference<0.001) yet not in the primary (p-value=0.99), secondary (p-value=0.63), or antral follicle counts (p-value=0.48). Mature and total follicle count meta-analyses were too few to be assessed.

Follicle count and type of animal

Subgroup analysis based on the type of animal showed no difference between C57BL/6J mice, ICR rats, Wistar rats, and Sprague Dawley rats for primary (p-value of subgroup difference=0.16) and antral follicle counts (p-value of subgroup difference=0.84). Subgroup analysis was not applicable to mature and total follicle counts. However, significant differences were observed for primordial and secondary follicle counts (p-value of subgroup difference=0.005 and 0.01), with Swiss albino rats showing a more marked improvement in primordial (SMD, (95CI), p-value; 7.22, (4.12,10.32), <0.01) and secondary follicle counts (SMD, (95CI), p-value; 8.65, (5.01, 12.29), <0.01).

Follicle count and study quality

All the studies in the primordial, primary, and secondary follicle count meta-analyses had low quality, yet for the antral follicle count no significant difference between high and low-quality papers was evident (p-value=0.28).

Mature and total follicle count meta-analyses were too few to be assessed.

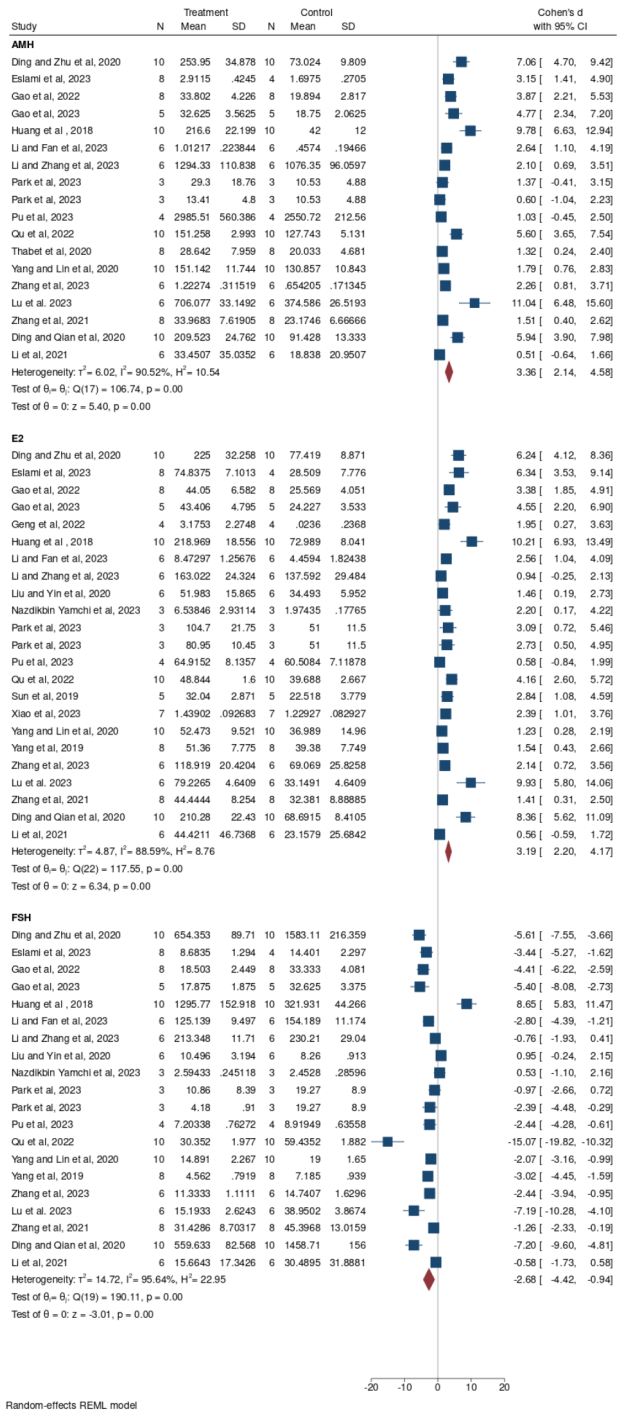

Fig. 3 Meta-analysis of Serum Hormones (Estradiol (E2), Anti-mullerian hormone (AMH), and Follicle stimulating hormone (FSH)) after MSC-EV treatment

Serum FSH, E2, AMH, and LH concentrations

Among the studies investigating serum hormones, 5 studies used an ELISA kit (CUSABIO, Wuhan, China) [20, 36, 41, 44, 60], 3 used ELISA kit (Enzyme-Linked Biotechnology, Shanghai, China) [45, 48, 52], one study assessed used ELISA kit (Cusabio, Cat no. CSBE06871m, CSB-E05109m, and CSB-E13156m, respectively, TX, USA) [62, 63], one study measured FSH using ELISA kit (Cat no: 334-096-4, Monobind), LH using ELISA kit (Cat no: 0234−96, Monobind), and E2 using ELISA kit (Cat no: 4925−300 A, Monobind) [49], one study measured FSH using ELISA kit (E-EL-R0391c, Elabscience, Houston, TX, USA), E2 using ELISA kit (E-OSEL-R0001, Elabscience), and AMH using ELISA kit (E-EL-R3022, Elabscience) [51], and one study measured FSH using ELISA kit (E-EL-R0026c, Elabscience, China), E2 using ELISA kit (E-EL-0152c, Elabscience, China), and AMH using ELISA kit (E-EL-R3022, Elabscience, China) [50]. ELISA kits (Mybiosource, USA;) were used by two studies [40, 46], (R&D Quantikine, R&D Systems Inc., Minneapolis, MN, USA) was used by one study [61], (MEIMIAN, China) was used by one study [56],

In one study the hormone levels were monitored by enzyme-linked immunosorbent assay kits (ImmunoWay, USA) [47], and in 6 studies serum hormone levels were measured but the kit being used was not mentioned [37, 39, 43, 54, 57, 64].

The mean concentration of FSH was evaluated by 19 studies [20, 36, 37, 39, 40, 45, 46, 48–56, 60, 62], the pooled results of which showed a decrease in FSH concentrations after treatment (SMD, (95CI), p-value; -2.68, (-4.42, -0.94), <0.001) with significant heterogeneity (I^2=95.64%, Fig. 3) when comparing 132 MSC-EVs treated animals to 136 untreated ones. The mean concentration of E2 was evaluated by 22 studies, the pooled results of which showed an increase in E2 concentrations after treatment (SMD, (95CI), p-value; 3.19, (2.20, 4.17), <0.001) with significant heterogeneity (I^2=88.59%) when comparing 148 MSC-EVs treated animals to 152 untreated ones. The mean concentration of AMH was evaluated by 17 studies, the pooled results of which showed an increase in AMH concentrations after treatment (SMD, (95CI), p-value; 3.36, (2.14, 4.58), <0.001) with significant heterogeneity (I^2=90.52%, Fig. 3) when comparing 123 MSC-EVs treated animals to 127 untreated ones. Publication bias assessed using Egger's regression test showed significant publication bias for all the above analyses yet al.l of them remained stable after sensitivity analysis using the leave-one-out method (Supplementary material 1, Figure S13-S18).

4 studies assessed serum LH levels, with Li et al. [39] finding a significant reduction when comparing 6 treated mice to 6 untreated ones, Xing et al. [47] finding a significant reduction comparing 6 treated ones to 6 untreated

ones, and Yang and Lin et al. [48] also finding significant reductions in LH when comparing 10 treated ones to 10 untreated ones. In contrast, Nazdikbin Yamchi [49] found no significant change when comparing 4 treated ones to 4 untreated ones. No meta-analysis was viable for this outcome. It should be noted that none of the included studies assessed the hormone levels while noting the phase of the estrous cycle at the time of evaluation.

Table 3 Results of the subgroup analysis of the serum hormones Meta-analyses based on EV source and administration route

Meta-analysis	EV source	Number of animals (number of studies)	SMD (95CI)	Administration route	Number of animals (number of studies)	SMD (95CI) I^2
Serum FSH	E-AFMSC	6 (1)	0.53 (-1.10, 2.16)	Intra-ovarian	106 (7)	-1.20 (-4.86, 2.47) 97.32%
	H-AFMSC	40 (2)	0.71 (-14.83, 16.24)			
	H-BMSC	6 (1)	-0.97 (-2.66, 0.72)			
	H-cMSC	12 (1)	-3.44 (-5.27, -1.617)	Intraperitoneal	44 (3)	-3.03 (-6.71, 0.64) 94.56%
	H-UCMSC	156 (11)	-4.01 (-6.13, -1.89)			
	iPSC-MSCs	12 (1)	-2.44 (-5.54, -2.17)			
	M-BMSC	20 (1)	-2.07 (-3.16, -0.99)	Intravenous	118 (8)	-3.51 (-5.64, -1.38) 92.76%
	Menstrual blood	16 (1)	-1.26 (-2.34, -0.19)			
	H-AFMSC	40 (2)	0.71 (-14.83, 16.24)			
Serum AMH	H-BMSC	6 (1)	1.37 (-0.41, 3.15)	Intra-ovarian	116 (7)	4.07 (1.74, 6.39) 93.57%
	H-cMSC	12 (1)	3.15 (1.41, 4.90)			
	H-UCMSC	128 (9)	3.93 (2.10, 5.77)			
	iPSC-MSCs	12 (1)	2.26 (0.81, 3.71)	Intraperitoneal	44 (3)	4.09 (-2.04, 10.22) 97.95%
	M-AFMSC	16 (1)	1.32 (0.24, 2.40)			
	M-BMSC	20 (1)	1.79 (0.76, 2.83)			
	Menstrual blood	16 (1)	1.51 (0.40, 2.62)	Intravenous	90 (6)	2.70 (1.90, 3.49) 62.53%
	H-AFMSC	40 (2)	7.67 (3.92, 11.42)			
Serum E2	H-cMSC	12 (1)	6.34 (3.53, 9.14)	Intra-ovarian	120 (8)	4.02 (1.70, 6.33) 93.72%
	H-UCMSC	170 (12)	3.0 (1.85, 4.14)			
	iPSC-MSCs	12 (1)	2.14 (0.72, 3.56)			
	M-BMSC	20 (1)	1.23 (0.28, 2.19)	Intraperitoneal	44 (3)	3.59 (-1.98, 9.16) 0%
	H-BMSC	16 (2)	2.93 (1.51, 4.33)			
	Menstrual blood	16 (1)	1.41 (0.31, 2.50)			
	H-AFMSC	48 (3)	6.69 (1.28, 12.13)	Intravenous	136 (10)	2.70 (1.90, 3.49) 7%
	E-AFMSC	6 (1)	2.2 (0.17, 4.22)			

Serum hormones and administration route

Subgroup analysis showed no significant difference between intravenous, intraperitoneal, and intra-ovarian administration regarding the FSH, E2, and AMH meta-analyses (Table 3).

Serum hormones and administration frequency

Meta-regression showed no significant association between administration frequency and the FSH meta-analysis (coefficient= -0.0191671, R2=0, explained variance p-value=0.924), the AMH meta-analysis (coefficient= -0.0425975, R2=0, explained variance p-value=0.751), and the E2 meta-analysis (coefficient= -0.0837977, R2=0, explained variance p-value=0.379).

Serum hormones and dose

Meta-regression showed no significant association between dose and FSH values (coefficient= -0.0073845, R2=0, explained variance p-value=0.306), E2 values (coefficient= -0.0013223, R2=0, explained variance p-value=0.747), and AMH values (coefficient=0.0029529, R2=0, explained variance p-value=0.729).

Serum hormones and EV source

Subgroup analysis regarding EV source showed significant differences in the FSH, E2, and AMH meta-analyses (Table 3), with H-UCMSC, H-AFMSC, and H-AFMSC performing the best in each meta-analysis, respectively.

Serum hormones and induction model

Subgroup analysis by the type of induction model showed no difference in the FSH (p-value=0.87), E2 (p-value=0.75), and AMH (p-value=0.75) meta-analyses.

Serum hormones and animal type

Subgroup analysis by animal type indicated significant differences in FSH, E2, and AMH level changes (p-value of subgroup difference for all <0.01). Regarding E2 levels, Swiss albino rates showed the highest increase in E2 (SMD, (95CI), p-value; 9.93 (5.80, 14.06), <0.01), while Sprague Dawley rats showed the least increase (SMD, (95CI), p-value; 1.16 (0.52, 1.80), <0.01). Other animal types were statistically comparable in this regard. Regarding AMH levels, Swiss albino (SMD, (95CI), p-value; 11.04 (6.48, 15.60), <0.01) and ICR rats (SMD, (95CI), p-value; 9.78 (6.63, 12.94), <0.01) showed the most increase, while Sprague Dawley rats showed the least increase (SMD, (95CI), p-value; 1.47 (0.90, 2.04), <0.01). Wistar rats and C57BL/6J mice were comparable. Regarding FSH levels, Swiss albino rats showed the most decrease (SMD, (95CI), p-value; -7.19 (-4.10, -10.28), <0.01), while Wistar rats showed the smallest decrease (SMD, (95CI), p-value; -7.10 (-22.39, 8.18), 0.36). Other animal types were statistically comparable in this regard.

Serum hormones and study quality

Subgroup analysis by study quality showed no difference in the FSH and AMH meta-analyses, yet high quality studies found significantly higher levels of E2 after treatment compared to low quality studies (high quality: SMD (95CI=7.37 (5.39, 9.35) vs. low quality: SMD (95CI=2.78 (1.91, 3.65).

Pregnancy and offspring

The main parameter for fertility in the animals was comparing the number of animals that got impregnated or the number of offspring the impregnated animals produced. the number of offspring was evaluated in 9 studies [21, 36, 41, 45, 46, 49, 53, 56, 62] which showed a significant increase in the number of offspring after treatment (SMD, (95CI), p-value; 3.70, (2.17, 5.23), <0.001) with high heterogeneity (I^2=88.64%) when comparing 73 MSC-EVs treated animals to 73 untreated ones (Fig. 4). The number of pregnant animals after treatment was evaluated in 7 studies [38, 41, 46, 49, 54, 56, 62], the pooled results of which showed an increased odds of pregnancy after MSC-EVs treatment (OR, (95CI), p-value; 10.25, (4.29, 24.46), <0.001) with low heterogeneity (I^2=0%) when comparing 73 MSC-EVs treated animals to 73 untreated ones (Fig. 5). Sensitivity analysis using the leave-one-out method showed both analyses to be stable and significant publication bias was only evident in the number of offspring analysis (Supplementary material 1, Figures S19-S22). Subgroup analysis and meta-regression was not viable for this outcome due to the low number of included studies.

Quality assessment

The quality of the included studies was measured using the SYRCLE tool, which showed a moderate risk of bias across most included studies, with lack of information regarding allocation concealment and blinding being the main concerns for bias. 1 study had high quality, while 28 studies had low quality, respectively (Figs. 6 and 7).

Discussion

Our collective findings suggest that MSC-EVs exhibit promising therapeutic potential in the management of infertility in POI in animal models. This potential is evidenced by their ability to ameliorate POI through the prevention of follicle loss, with our study showing an increase in the total number of follicles, primordial follicles, secondary follicles, mature follicles, and antral follicles, as well as the modulation of hormonal profiles characterized by decreased FSH levels and increased E2 and AMH levels. This improvement also translated

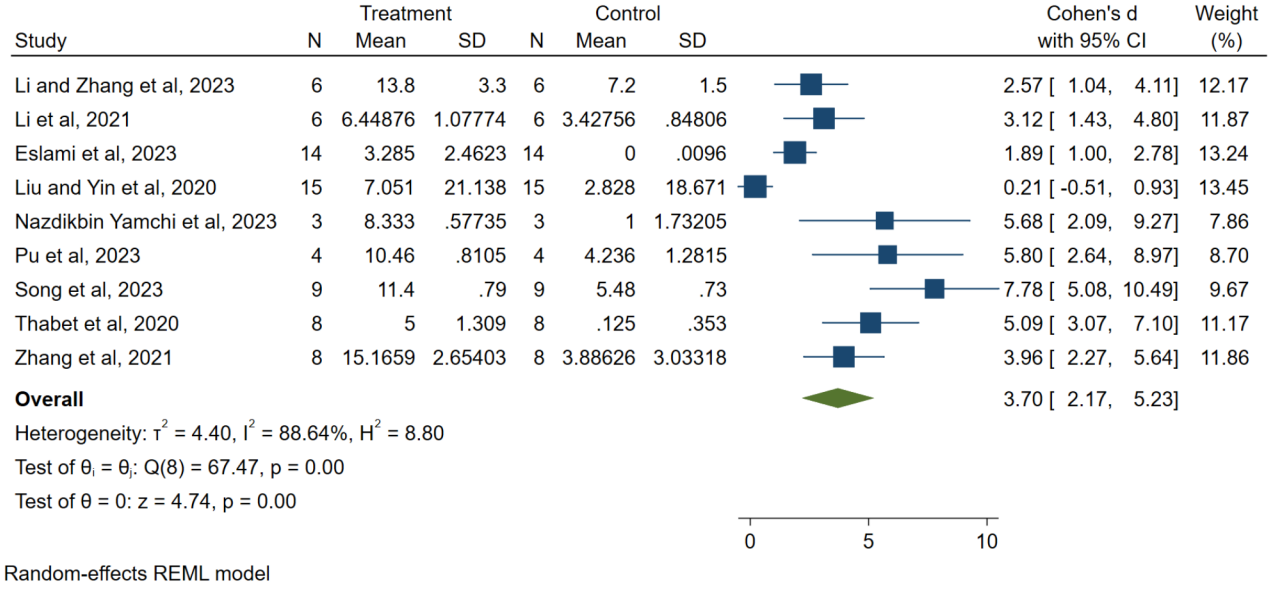

Fig. 4 Meta-analysis of the mean number of offspring after MSC-EV treatment

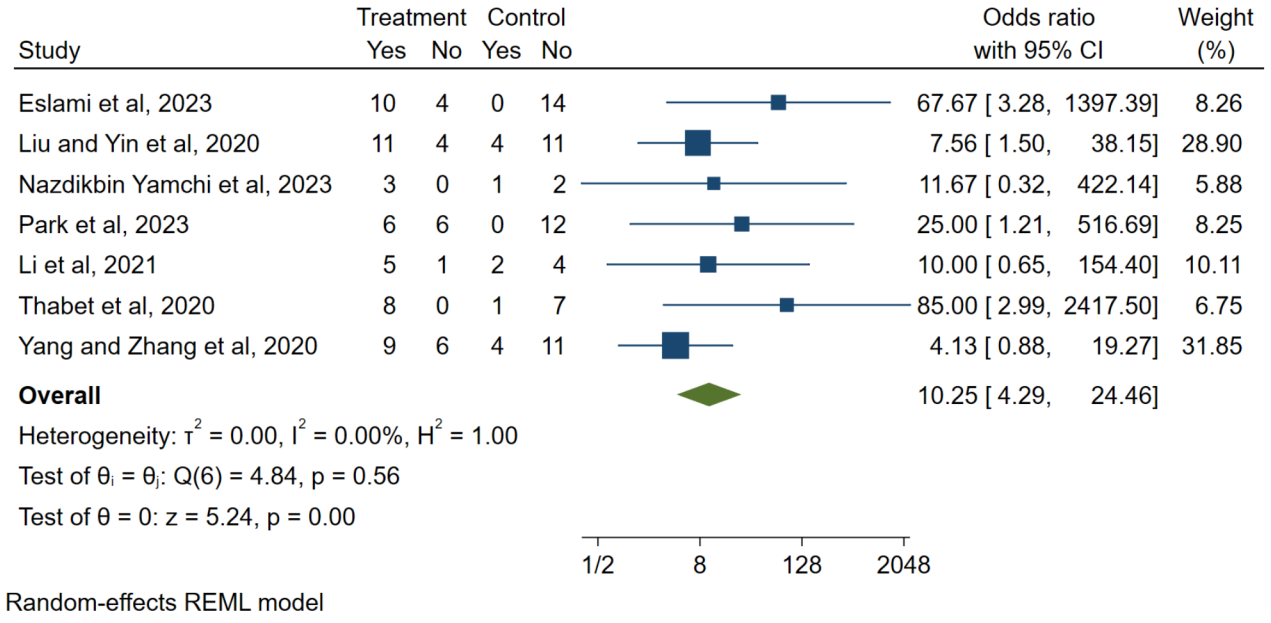

Fig. 5 Meta-analysis of the number of pregnant animals after MSC-EV treatment

to enhancement of fertility outcomes, as indicated by improvements in the number of offspring and the odds of pregnancy in the treated animals (Fig. 8). The significance of these findings lies in the absence of enhanced fertility from other treatments that address the underlying mechanisms. Existing approaches for infertility in POI predominantly center on assisted reproductive technology, which has shown limited promise. Moreover, the hormonal variations identified in our study may serve as a robust alternative to hormone replacement therapy (HRT) for POI patients, being potentially superior to HRT, primarily a palliative measure, as it fails to improve ovarian function [65]. The elevated estrogen values noted in our research may yield positive effects on the cardiovascular system, bone health, and overall well-being of patients [66]. Overall, these outcomes imply a potential translational application of this innovative approach for the treatment of human POI.

In the reviewed studies, several miRNAs and their downstream pathways were identified as potential

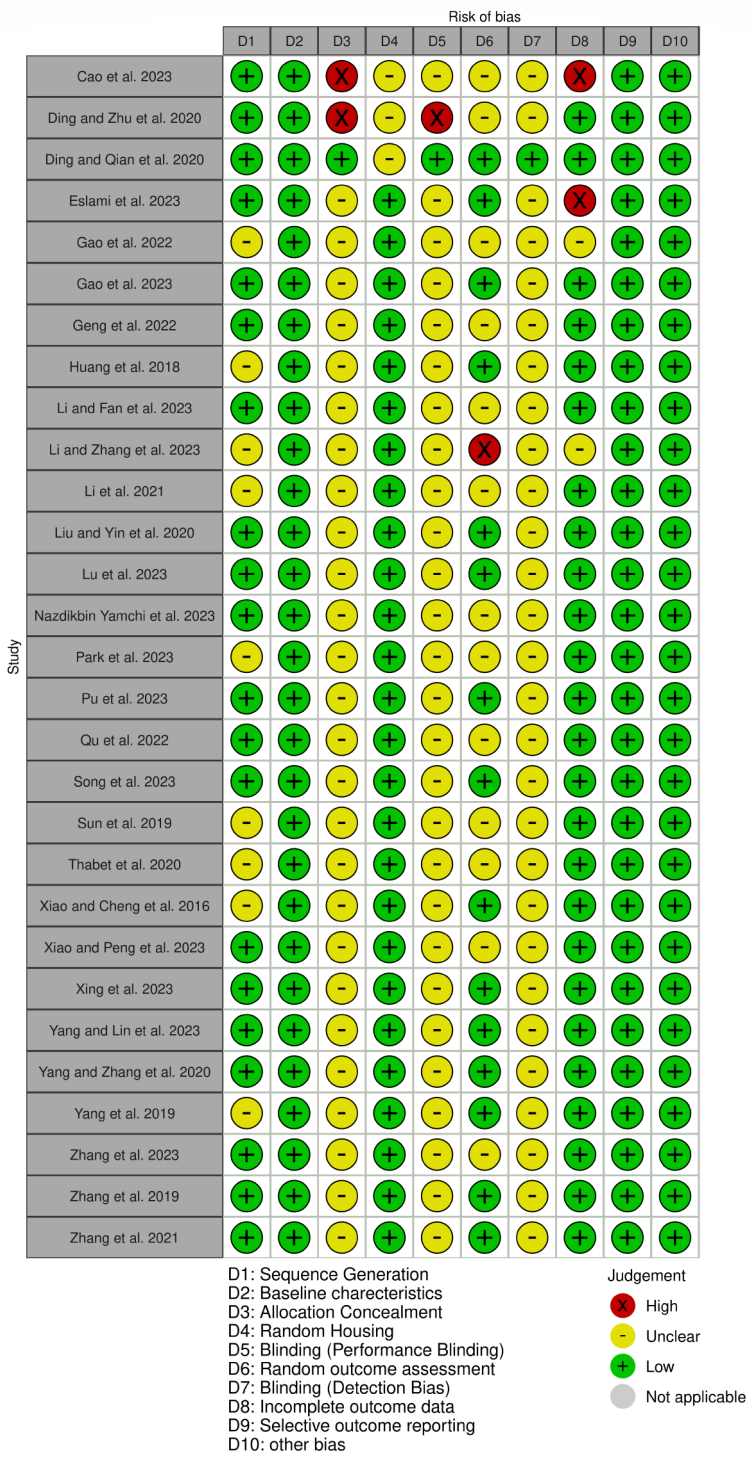

Fig. 6 The risk of bias for each study in each domain of the SYRCLE tool

mechanisms of action. Among these pathways is the ILK-PI3K/AKT pathway mentioned in 9 of the included studies, wherein MSC-EVs were found to contain miRNAs that upregulate ILK and its downstream pathway PI3K/AKT. This leads to decreased apoptosis, enhanced proliferation, and increased viability of granulosa cells [59]. Another potential target of EV therapy is the sirtuin family, comprising seven enzymes (SIRT1-7). EV therapy has been shown to downregulate SIRT4, thereby reducing oxidative stress and alleviating POI [37]. Additionally,

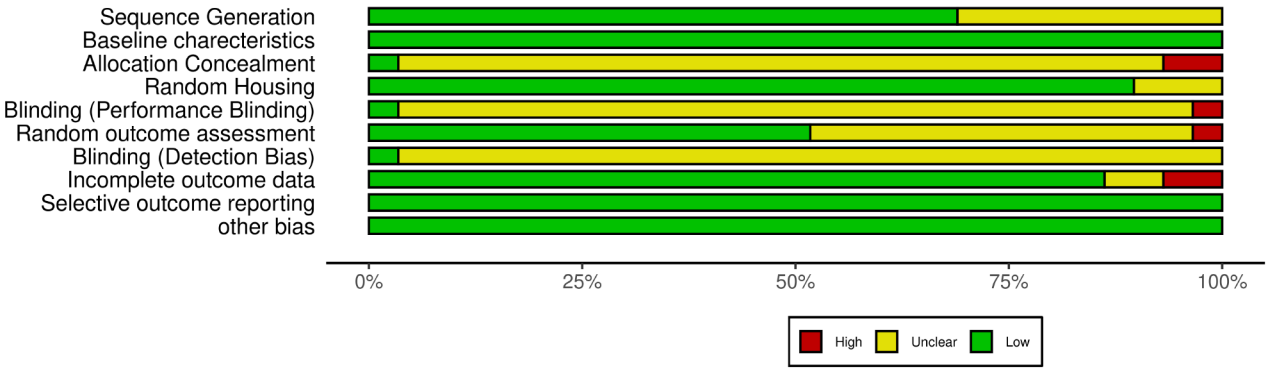

Fig. 7 Summary of the risk of bias in every domain of the SYRCLE tool

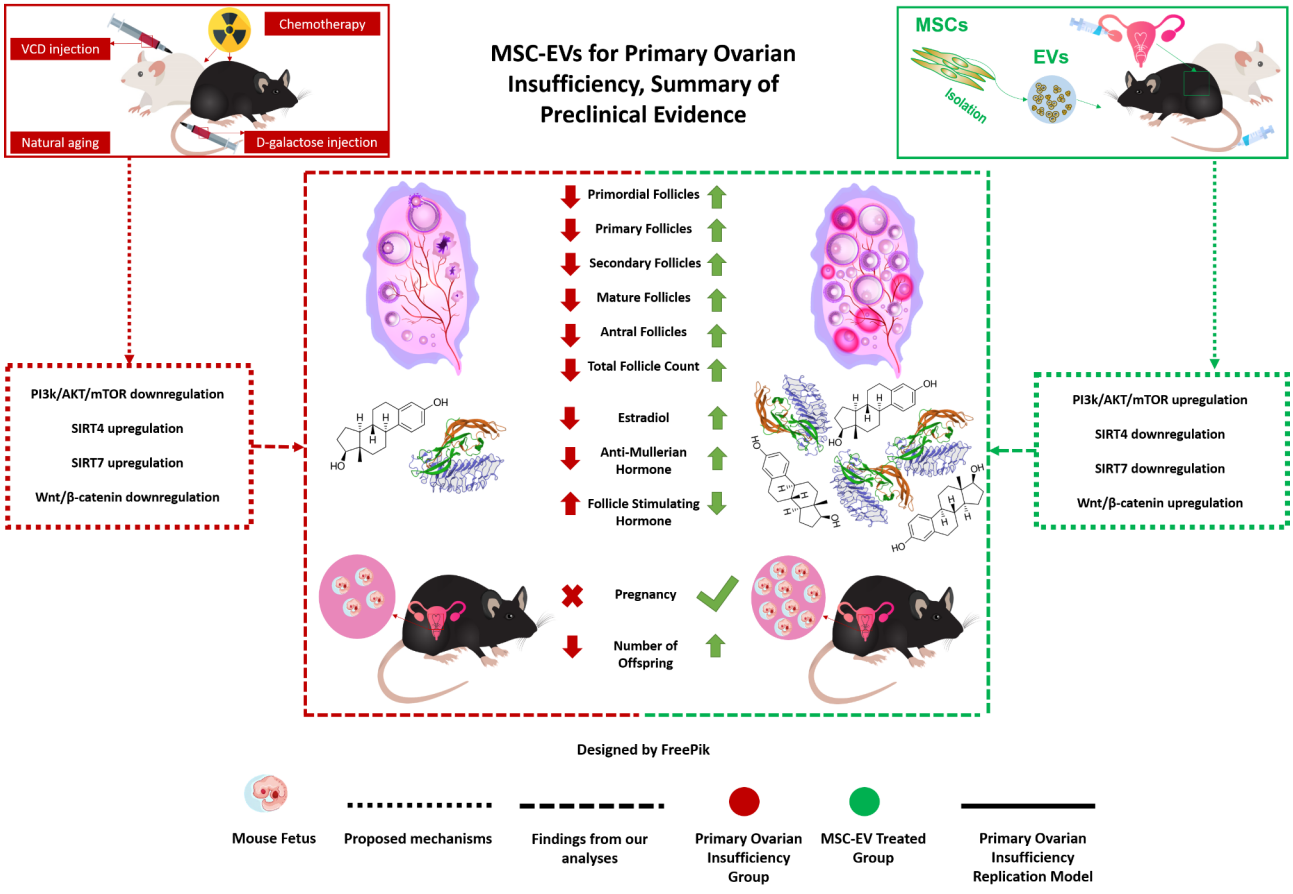

Fig. 8 Graphical abstract

miRNAs present in EVs can suppress SIRT7 and its downstream molecules, exerting effects on POI through this pathway [55]. Furthermore, HMG-box transcription factor 1 (HBP1) emerges as another target of EV therapy discussed in the studies. The cargo of H-UCMSC derived EV, miR-29a, was found to downregulate HBP1, consequently activating the Wnt/β-catenin pathway and restoring ovarian function [52, 67]. Overall, it appears MSC-EVs utilize numerous pathways, most notably the PI3K/AKT pathway, to ascertain their therapeutic effect.

A meta-analysis assessing the effects of H-UCMSC on POI deduced that MSC therapy can improve estrous cycle, abbreviate the estrous cycle, augment folliculogenesis, enhance E2 and AMH levels, and diminish FSH and LH levels [68]. Another meta-analysis investigating the efficacy of stem-cell therapy on POI scrutinized

both animal and clinical investigations. Animal studies revealed analogous outcomes as aforementioned, in addition to heightened fertility in the treated cohort. while clinical studies unveiled reduced FSH levels, elevated antral follicle count levels, and an increased likelihood of pregnancy and live birth in patients undergoing MSC therapy [69]. Another promising modality involves the utilization of exosomes derived from MSC, which is the focal point of our review. A meta-analysis exploring the effects of MSC-EVs on female reproductive disorders, including intrauterine adhesions and POI, concluded that AMH levels are elevated in animal models of POI when subjected to MSC-EVs treatment [70]. Our findings align with the results of both animal and clinical investigations discussed. This concurrence between the outcomes of MSC-EVs therapy and MSC therapy suggests that exosomes may be equally efficacious as MSC. However, there remains a dearth of comparative studies comparing the use of MSC with MSC-EVs, thus rendering the relative effectiveness of each approach unknown. Future investigations are warranted to bridge this gap.

MSCs can be derived from various sources, yet a definitive standard determining the optimal source for treatment of POI remains elusive. Among the sources explored, H-UCMSCs have been prominently featured in the studies reviewed. Unique advantages of umbilical cord-derived MSCs include their diverse sourcing options, ease of procurement, and reduced likelihood of eliciting an immune response [67], it was also the best EV source exerting its effects on primary follicles. The mechanism of this improved performance is yet to be determined. Another notable mesenchymal stem cell source investigated in certain studies are H-AFMSCs, which possess low immunogenicity, have demonstrated prolonged survival in POI mice, and secrete a spectrum of growth factors [57], our subgroup analysis revealed greates impact of this EV source on secondary and antral follicles which may be attributed to its secretion of these diverse growth factors. Recently, human menstrual blood-derived MSCs have emerged as a potential MSC source for POI treatment due to their non-invasive acquisition method, high proliferative capacity, and lack of ethical concerns [21, 71], Inversely, the impact of this EV source was poorest on primordial follicle count in our subgroup analysis. However, these human-derived MSCs have limitations such as the requirement for invasive retrieval and limited in vitro proliferative potential. In contrast, iPSC-MSCs exhibit the ability to differentiate into various cell types, offer ease of procurement, and have the potential for indefinite expansion, with prior studies highlighting thier potential [59]. While numerous other sources of MSCs have been investigated including both human and murine, a comparative analysis to determine their efficacy in POI treatment is lacking. In our subgroup analysis based on EV source, AMH levels exhibited a discernible difference in favor of H-AFMSC and H-ESC with the subsequent meta-regression finding an $R_2 = 0.2$, indicating a low level of correlation. Nonetheless, due to the limited number of studies utilizing these sources, it is not possible to confidently interpret this disparity and future research endeavors should aim to address this gap in knowledge by conducting head-to-head comparisons of these MSC sources.

Our subgroup analysis regarding the route of EV administration revealed no significant effect on hormone levels or follicle counts. Previous studies have employed intravenous and intra-ovarian injections to treat infertility in polycystic ovary syndrome, both yielding successful outcomes in inducing fertility, however, intravenous treatment demonstrated greater potency in the metabolic effects of EV therapy, while the intra-ovarian route showed higher efficacy in restoring fertility [72]. Another administration route investigated in other studies is intraperitoneal injection, yet insufficient data exist to determine its comparative potency. Further comparative studies are warranted to elucidate the diverse effects of various administration routes in EV therapy.

The variability observed in both the dosage and frequency of EVs administration across the studies included in our review prompts consideration of its potential impact on therapeutic outcomes in disease management. Despite this inherent heterogeneity, our meta-regression analyses revealed no statistically significant association between either the dosage or frequency of EV administration and follicle count or serum hormone levels. However, it is imperative to exercise caution in the interpretation of these findings, given the considerable diversity in EV quantification methodologies and the absence of standardized protocols for EV preparation. Consequently, a recommendation emerges to adopt more qualitative approaches in assessing EV preparations, focusing particularly on the efficacy and functional attributes of the EV cargo [73].

In our review, various animal models have been utilized to replicate the pathophysiology of POI. The predominant model employed involves the induction of POI through chemotherapy. Chemotherapeutic agents have been observed to disrupt menstrual cycles and reduce ovarian size, follicle count, oocyte quantity, and embryo development. Consequently, this leads to diminished ovarian reserve, impaired ovulation, and reduced fertilization rates [25]. The mechanism by which chemotherapy induces ovarian dysfunction involves direct targeting of primordial follicles as well as indirect effects on growing follicles, ultimately impacting primordial follicle reserve. Furthermore, chemotherapeutic agents can induce oocyte apoptosis by causing damage to both the oocyte itself and the surrounding somatic cells. Cell death

within the ovary induced by chemotherapeutic agents may occur via necrosis, apoptosis (involving the ceramide pathway), or atresia [74], Interestingly this model showed to be significantly altering better when considering the primordial follicle count. This greater effect may be supported by the fact the primordial follicles are a primary target of chemotherapy. Another model utilized in certain studies involves the induction of premature ovarian aging using D-galactose. Classical galactosemia, a hereditary disorder, is recognized as a causative factor for ovarian failure. Subsequent investigations have utilized this hypergalactosemic state to induce POI in animal models. This model offers the advantage of encompassing both follicle depletion and dysfunction, characteristics commonly observed in POI. Thus, it is proposed that this model can effectively simulate the diverse etiologies of POI [75, 76]. Additionally, another model employed to investigate POI involves the use of 4-vinylcyclohexene diepoxide. This metabolite induces ovarian damage and diminishes primordial and primary follicle populations in animal models. The deleterious effects of 4-vinylcyclohexene diepoxide are believed to be mediated through various apoptotic signaling pathways, including the B-cell lymphoma 2 (Bcl-2) pro-apoptotic signaling pathway, kit ligand signaling, the rapamycin-insensitive companion of mammalian target of rapamycin (rictor)/mTORC2 pathway, and the nuclear factor erythroid 2-related factor 2-mediated oxidative stress response pathway [77].

Our study is constrained by its own limitations. First, significant variations exist in the methodology of the included studies, including the dosing, type MSC, mode of EV acquisition, administration frequency, timing of treatment, and administration route. These variations may impact the outcomes. However, previous studies have determined that the administration route does not affect these outcomes [68]. Nonetheless, the head-to-head comparison between different administration routes, EV sources, and other methodologies needs to be conducted in future research. Second, many of the included studies did not provide detailed characterization of the MSC-EVs they utilized, which makes it challenging to ascertain which components of the MSC-EVs are responsible for the therapeutic effects. This lack of information in turn restricts the standardization and optimization of MSC-EVs for usage in clinical research. Third, the model of POI varies among reports. This discrepancy could alter the underlying disease mechanism and consequently, the therapeutic effects. Nevertheless, it is believed that regardless of the cause, the pathophysiology of POI remains the same [68]. Third, most studies focused on short-term outcomes, with limited data on the long-term safety and efficacy of MSC-EVs in POI treatment. This fact limits the future translation of this preclinical research into clinical studies. We suggest future studies conduct more long-term studies, with a primary focus on safety in order to set the groundwork for future clinical applications. Fourth, publication bias was evident in all of our outcomes, severely undermining the grade of our evidence. This may imply some studies that are not achieving results in favor of MSC-EVs therapy are not getting published, which can be remedied by increasing sample sizes or by studying the biodistribution of MSC-EVs, alleviating concerns over faulty methodologies. Fourthly, high heterogeneity was observed in some of our outcomes, potentially undermining the robustness of our results. However, this heterogeneity can be contributed to the EV source and induction models used. Different follicle counting may have also contributed to heterogeneity, with studies summing more sections better representing the actual count of follicles. We suggest future studies to assess follicle counts using such methods. Hormonal assessment should also be conducted with consideration of estrous cycle phases, another limitation of our review and all of the included studies. Another limiting factor was the quality of included studies which was generally low. Despite this low quality, our analysis revealed no difference of follicle counts between low- and high-quality studies and hormone levels with the exception of E2 levels. As a result, the impact of this quality assessment on the outcomes observed is not concerning, however future high-quality studies are required to confirm our findings.

Conclusion

In conclusion, this meta-analysis underscores the promising therapeutic potential of MSC-EVs in treating POI, as it targets the underlying disease mechanisms and improves follicle count and hormonal changes, culminating in improved fertility outcomes in animal models of POI. This review highlights the evidence of the effectiveness of MSC-EVs modality in the preclinical stage. However, these results should be interpreted with caution as significant publication bias and heterogeneity does not allow for definite conclusions. Additionally, safety evaluations and the translation of our findings to clinical approaches exceeds the scope of our study and requires further investigation.

Abbreviations

POI	Primary ovarian insufficiency
FSH	Follicle stimulating hormone
AMH	Anti-müllerian hormone
OCP	Oral contraceptives
MSCs	Mesenchymal stem cells
MSC-EVs	Mesenchymal stem cell-derived extracellular vesicles
EVs	Extracellular vesicles
E2	Estradiol
LH	Luteinizing hormone
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
SMD	Standardized mean difference

OR	Odds Ratio
H-UCMSC	Human umbilical cord mesenchymal stem cells
H-AFMSC	Human amniotic fluid mesenchymal stem cells
iPSC-MSCs	Induced pluripotent mesenchymal stem cells
H-BMSC	Human bone marrow mesenchymal stem cells
M-AFMSC	Murine amniotic fluid mesenchymal stem cells
M-BMSC	Murine bone marrow mesenchymal stem cells
H-cMSC	Human clonal mesenchymal stem cells
E-AFMSC	Ewe amniotic fluid mesenchymal stem cells
HRT	Hormone replacement therapy
HBP1	HMG-box transcription factor 1

Supplementary Information

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Supplementary Material 1

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