Effects of Stem Cell Derived Exosome Therapy on Erectile Dysfunction: A meta-analysis of preclinical studies

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Research Article

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Abstract

Background: Exosome is considered a potential cell-free therapy for treating erectile dysfunction (ED), which was investigated in some preclinical studies. The therapeutic efficacy has not been comprehensively evaluated.

Aim: To further study the therapeutic effects of exosomes derived from stem cells on ED in preclinical studies and investigate some possible factors that influence treatment efficacy.

Methods: The literature research was conducted in Web of Science, PubMed and Embase to retrieve studies utilizing stem cell derived exosomes to treat ED. Revman 5.3 was used to perform subgroup analysis of intracavernosal pressure/mean artery pressure (ICP/MAP) and structural changes. Publication bias was assessed with Egger's test, funnel plot, and sensitivity analysis by Stata 15.0.

Outcomes: The ICP/MAP and structural changes after stem cell treatment.

Results: Of 146 studies retrieved, 11studies are eligible. Pooled analysis showed that stem cell derived exosomes ameliorates damaged ICP/MAP (WMD 3.68; 95% CI 2.64-4.72; P < .001) and structural changes, like the ratio of smooth muscle to collagen (SM/Collagen), alpha smooth muscle actin (α-SMA), the cluster of differentiation 31 (CD31), neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS), transforming growth factor-β1 (TGF-β1) and caspase-3 protein expression. Subgroup analysis indicated that exosome type and ED model type make no difference to curative effects.

Conclusion: This meta-analysis suggests the efficacy of stem cell derived exosome therapy for ED and the possible mechanism in erection restoration and structure renovation.

Introduction

Erectile Dysfunction (ED) refers to the impotence to obtain or maintain an erection enough to permit satisfactory sexual intercourse.[1]The incidence grows with age, especially in men aged over 40 years and it affects the quality of life causing physiological and psychological problems.[2] ED is an important complication in men with diabetes mellitus for its multifactorial pathophysiology and more attention has been focused on post-radical prostatectomy erectile dysfunction (pRP-ED) due to the growing incidence of prostate cancer (PCa) in line with increasing male life expectancy.[3] Many other factors are reported to be involved with ED including cardiovascular diseases, metabolic syndrome, neuropathic damage, lower urinary tract symptoms, Peyronie's disease (PD), obstructive sleep apnea (OSA), psychiatric disorders and the coronavirus disease 2019 (COVID-19).[4–7]

In terms of therapies and management, oral phosphodiesterase type 5 inhibitors(PDE5Is), such as sildenafil and tadalafil,[8,9] were regarded as the first-line treatment of erectile dysfunction.[4] Other treatment modalities include intra-cavernous (IC) injection therapies, testosterone therapy, vacuum constrictive devices, and penile prostheses. In addition, published literature shows low intensity extracorporeal shock wave therapy (LIESWT) and low intensity pulsed ultrasound (LIPUS) therapy to improve erectile function and penile hemodynamic by inducing neovascularisation and promoting tissue regeneration.[10,11] However, they are far from flawless. For example, PDE5Is have less efficacy on organic ED. Vacuum constrictive devices are expensive and unnatural erections cannot meet satisfactory of patients. The equipment cost of LIESWT is relatively high, and the actual physiological changes of penile tissue and the long-term risk of shock waves and other factors have not been fully elucidated. Therefore, there is still a great need for comparatively more effective therapeutic methods that can provide long-lasting improvement for erectile dysfunction. By researching relevant studies, we found exosomes derived from stem cell may provide a novel promising therapeutic strategy.

Exosomes refer to a class of extracellular vesicles (EVs) with a diameter of 50 to 100 nm, which are secreted by almost all cells.[12] They usually encapsulate a complex payload containing lipids, signaling proteins, nucleic acids, thus enabling cells to exchange information for multiple physiologic and pathologic functions.[13] Accordingly, the beneficial effects of exosomes on ED in rat models have been found in recent exploratory treatment experiments.[14,15] Among these studies, exosomes are mostly derived from stem cells, including bone marrow-derived mesenchymal stem cell (BMSC), adipose-derived mesenchymal stem cell (ADSC) and human urine-derived stem cells. However, the value of stem cell derived exosomes in ED treatment has not been comprehensively investigated yet. The aim of this work was to systematically review and meta-analyses the evidence on the treatment efficacy of exosomes derived from different stems cells in various ED rat models.

Methods

Literature search Strategy and Selection criteria

This meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis.[16]

Comprehensive literature searches for studies published between January 1, 2017 February 28, 2022 were conducted in PubMed, Web of science and EMBASE for pertinent studies. We applied the preprint database to find potential articles without peer review to avoid publication bias.

The key words are as follows: (“stem cell” or “SC”) and (“exosomes” or “extracellular vesicles”) and (“erectile dysfunction” or “ED”). Additionally, we hand-searched the references of all relevant articles if necessary. We did not apply any language restrictions. Reviews, duplicates, conference abstracts and clinical trials were excluded. Abstracts were screened for relevance, and the full texts were read when it was unclear from the abstract.

The inclusion criteria were as follows:

1. Randomized/nonrandomized controlled animal experiment.

2. Rat/mouse model.

3. The utilization of exosomes to improve erectile function

Quality Assessment

Two investigators were assigned to separately assess the methodological quality of included studies. The ARRIVE criteria[17] and ESSM guidelines[18] for reporting ICP/MAP were applied in our assessment standards. There are 27 criteria: one point for each criterion (not mentioned or unclear, 0 point; yes, 1 point). Studies with score ≥18 were considered high quality and studies with score <18 were considered moderate quality.

Data Extraction

Data extraction from included studies were conducted independently by two authors of our team. Disagreement was resolved by referring to the original article. Moreover, a third author resolved the disagreements when necessary. The following information from each study was extracted: first author, year of publication, source of exosomes, exosomes indicators, ED model type, species, experiment animal population, follow- up time, injection frequency, injection methods, exosomes number and outcome indexes. The mean and standard error of mean (SEM) or standard deviation (SD) were extracted from the included article text. The software Web Plot Digitizer (https://automeris.io/WebPlotDigitizer/) was used to extract numerical values from charts if the results were only displayed as graphs or we failed to receive reply from the corresponding authors of the articles. Subsequently, the collected data were transferred manually into an Excel spreadsheet for data analysis.

Subgroup analyses of 3 factors were conducted: (i) exosome type: exosomes origination adopted in studies, ADSC vs BMSC vs Human Urine-Derived Stem Cells; (ii) ED model type: diabetic mellitus ED (DMED) vs cavernous nerves injury induced ED(CNI-ED) vs PD associated ED vs artery injury induced ED(AI-ED) and chronic intermittent hypoxia (CIH) induced ED resembling the OSA disease; (iii) the level changes of structure markers: the ratio of smooth muscle to collagen (SM/Collagen) vs the cluster of differentiation 31 (CD31) vs α-smooth muscle antibody(α-SMA) vs endothelial nitric oxide synthase (eNOS) vs neural nitric oxide synthase (nNOS) vs the apoptotic protein cleaved caspase-3 vs transforming growth factor-β1 (TGF-β1).

Statistical Analysis

We utilized the software RevMan 5.3 to analyze extracted data. Weighted mean difference (WMD) with 95% confidence intervals (CI) were used to show the difference of ICP/MAP between the exosomes therapy group and the ED control group. Standardized mean difference (SMD) with 95% CIs were applied as to structural changes in corpus cavernosum, including the ratio of SM/Collagen, CD31, α-SMA, eNOS, nNOS, TGF-β1 and caspase-3 protein expression. Heterogeneity was evaluated using the I^2 -statistic test. Random effects model was adopted if I^2 ≥50% and fixed effects model will be applicable if I^2 ≤50%. Stata 15.0 was used to examine publication bias with Egger's test,[19,20] funnel plot.

Results Study

Selection and Characteristics

As shown in Fig. 1, 146 publications were identified after research. We enrolled 11 studies after full-text review. Characteristics of eligible studies were described in Table 1.

Table 1

Characteristics of included studies

Year	First author	Producer cell	Isolation methods	exosomes labels	Exosome dose	ED Model	Species	Animal age(week)	Injection method	Frequency	Follow up(we
2017	FZ Chen[21]	ADSC	multistep centrifugation	CD63, CD81, calnexin	100μg	Diabetic	SD rats	6	IC injection	1	4
2021	Li Liang[22]	ADSC	ExoQuick-TC reagent	CD9, CD63, TSG101	400μg	Hypoxia	SD rats	not mentioned	IC injection	8	8
2020	JY Song[27]	ADSC, BMSC	multistep centrifugation	CD9, CD63, TSG101, calnexin	100μg	Diabetic	SD rats	8	IC injection	1	4
2018	M. Li[15]	BMSC, ADSC	PureExo Exosome isolation kit	CD63, HSP70, CD81	100μg	CNI	SD rats	12	IC injection	1	3
2020	Jian Wang[23]	ADSC	ultracentrifugation, ultrafiltration	CD31, CD9, CD63, CD81	200μg	Diabetic	SD rats	8	intravenous injection	1	2
2019	YZ Liu[26]	BMSC	multistep centrifugation	CD9, TSG101	50 or 100ug	AI	SD rats	12	IC injection	1	4
2018	Xi Ouyang[14]	BMSC	multistep centrifugation	CD63, TSG101, Flotillin-1	100μg	CNI	SD rats	10	IC injection	1	4
2021	Li liang[24]	ADSC	differential centrifugation	CD63, CD9	150μg in 100μL PDNPs-PELA gel	CNI	SD rats	6–8	IC injection	1	3
2017	L. L. Zhu[25]	ADSC	exosome precipitation solution, ExoQuick	CD63, CD9	10 or 100μg	Diabetic	SD rats	10	corpus cavernosum injection	1	4
2020	Qiyun Yang[28]	HUSC	ultracentrifugation, ultrafiltration	CD9, CD63, TSG101, Alix	100μg	PD	SD rats	not mentioned	Intratunical injection	1	4
2019	Bin Ouyang[29]	H USC	ultracentrifugation	CD63, calnexin	100μg	Diabetic	SD rats	not mentioned	IC injection	1	4

ADSC = adipose-derived mesenchymal stem cell; BMSC = bone marrow-derived mesenchymal stem cell; HUSC = human urine-derived stem cells; SD rat = Sprague Dawley rat; IC= intra- Intra-cavernous; CNI=cavernous nerve injury; PD= Peyronie's disease; AI refers to artery injury by bilateral internal iliac arteries ligation;

the column “exosome dose” means the amount of stem cell derived exosomes dissolved in PBS.

PDNPs-PELA =Polydopamine nanoparticles incorporated poly (ethylene glycol)-poly(ε-caprolactone-co-lactide)

Exosomes derived from adipose derived stem cell (ADSC-Exos) were applied in 5 studies[21–25] and bone marrow stem cell derived exosomes (BMSC-Exos) were used in 2 studies.[14,26] While both parental cells were utilized in another 2 studies.[15,27] The other 2 studies exploited exosomes derived from human urine-derived stem cells.[28,29]

As to ED models, 5 studies[21,23,25,27,29] injected streptozotocin (STZ) into animals to establish diabetic mellitus model. 3 studies[14,15,24] constructed neurogenic ED model by damaging cavernous nerves surgically.

Injection of TGF-β1 into rat tunica albuginea was utilized in one study to create PD model[28] and one research utilized CIH to mimic OSA induced ED.[22] Another study focused on artery injury indued ED.[26]

Quality Assessment

The quality score of 6 studies are ≥ 18 and the other 5 studies received < 18 points. More details were shown in Table 2. We also evaluated the risk of bias using SYRCLE's tool,[30] which is based on the Cochrane risk of bias tool and has been adjusted for aspects of bias that play a specific role in animal intervention studies.

Table 2
Quality assessment of included studies

		study design		sample size		inclusion and exclusion			Randomization			
Year	Study	Control	Experiment unit	Number	Calculation	Criteria	Exclusion	N	Randomization	Confounder	Blinding	outcome measure
2017	FZ Chen	1	1	1	0	1	0	1	1	0	0	1
2021	Li Liang	1	1	1	0	1	0	1	1	0	0	1
2020	JY Song	1	1	1	0	1	1	1	0	0	0	1
2018	M. Li	1	1	1	0	1	0	1	1	0	0	1
2020	Jian Wang	0	1	0	0	1	0	0	0	0	0	1
2019	YZ Liu	1	1	1	0	1	0	1	1	0	0	1
2018	Xi Ouyang	1	1	1	0	1	0	1	0	0	0	1
2021	Li liang	1	1	0	0	0	0	0	1	0	0	1
2017	L. L. Zhu	1	1	1	0	1	0	0	1	0	0	1
2020	QY Yang	1	1	1	0	1	0	1	1	0	0	1
2019	Bin Ouyang	1	1	1	0	1	1	1	1	0	0	1

		animals		procedures				results	ICP/MAP			cell	exosome	
year	study	species	further	what	when	where	why	summary	nommalization by MAP	images of traces	APO test	phenotype	label	morpholo
2017	FZ Chen	1	1	1	1	1	1	1	1	1	0	0	1	1
2021	Li Liang	1	0	1	1	1	1	1	1	1	0	1	1	1
2020	JY Song	1	0	1	1	0	1	1	1	1	1	1	1	1
2018	M. Li	1	0	1	1	0	1	1	1	1	0	0	1	1
2020	Jian Wang	1	0	1	1	0	1	1	1	1	0	1	1	1
2019	YZ Liu	1	0	1	1	0	1	1	1	1	0	0	1	1
2018	Xi Ouyang	1	0	1	1	0	1	1	1	1	0	1	1	1
2021	Li liang	1	0	1	0	0	1	1	1	1	0	0	1	1
2017	L. L. Zhu	1	0	1	1	0	1	1	1	1	0	0	1	1
2020	QY Yang	1	0	1	1	0	1	1	1	1	0	1	1	1
2019	Bin Ouyang	1	0	1	1	1	1	1	1	1	1	0	1	1

Meta-analysis

Intracavernosal Pressure/Mean Artery Pressure

The pooled analysis showed that stem cell derived exosome therapy increased ICP/MAP significantly (N = 194, MD 3.68, 95% CI [2.64–4.72]; Z = 6.95 (P < 0.01); $\chi 2 = 45.61$, I2 = 74%), which represents the improvement of erectile function (Fig. 2A).

The subgroup analysis of ICP/MAP was conducted based on 2 factors: (i) producer cell; (ii) ED model type.

First, there is no significant difference among different exosome types ($\chi2 = 0.72$, P = 0.70; ADSC N = 103, MD 3.80, 95% CI [2.20–5.40]; Z = 4.67(P < 0.01); $\chi2 = 26.46$, I2 = 77% vs BMSC N = 67, MD 4.23, 95% CI [1.81–6.65]; Z = 3.43 (P < 0.01); $\chi2 = 18.19$, I2 = 84% vs USC N = 24, MD 3.31, 95% CI [1.90–4.72]; Z = 4.6 (P < 0.01); $\chi2 = 0.24$, I2 = 0%) (Fig. 2A).

Second, the therapeutic effects don't vary depending on ED model types ($\chi2 = 7.84$, P = 0.10; DMED N = 86, MD 4.37, 95% CI [2.54–6.23]; Z = 4.68(P < 0.01); $\chi2 = 19.95$, I2 = 75% vs CNIED N = 76, MD 3.17, 95% CI [1.29–5.04]; Z = 3.31(P < 0.01); $\chi2 = 17.82$, I2 = 83% vs OSA-ED N = 12, MD 3.39, 95% CI [1.38–5.39]; Z = 3.31 (P < 0.01) vs AI-ED N = 12, MD 3.80, 95% CI [1.63–5.97]; Z = 3.43 (P < 0.01) vs PD-ED N = 8, MD 3.99, 95% CI [0.92–7.06]; Z = 2.54 (P < 0.01)) (Fig. 2B).

Structural Changes

To illustrate the underlying mechanism of exosome therapy for ED, structural changes were analyzed. Stem cell derived exosomes restored SM/Collagen, smooth muscle content, CD31, nNOS and eNOS damaged by ED resulting from different risk factors. Stem cell derived exosomes also ameliorated the damage of TGF β1 and caspase 3 induced in ED (Table 3).

Table 3
Subgroup analyses for structural changes

Biomarker	N	SMD	95% CI	Z	P value	χ^2	I^2
SM/collagen	144	3.71	[3.10,4.32]	11.92	< 0.001	12.91	38%
CD31	44	5.32	[3.86,6.78]	7.14	< 0.001	2.73	27%
a-SMA	82	3.57	[1.01,6.14]	2.73	0.006	36.3	86%
eNOS	58	3.27	[2.39,4.15]	7.32	<0.001	1.6	0%
nNOS	82	2.12	[0.22,4.03]	2.19	0.03	41.75	88%
TGF-β1	38	-4.3	[-7.17,-1.43]	2.94	0.003	8.48	76%
caspase3	32	-4.42	[-5.86,-2.99]	6.04	<0.001	0.56	0%

Bias Assessment

Egger's test (t = 10.77, P = 0.00) indicated publication bias in ICP/MAP analysis. The Egger's publication bias plot and funnel plot (Fig. 3) shows the consistent outcome.

Discussion

A total of 11 published preclinical studies were included in our analysis. Overall, our analysis suggests that stem cell derived exosomes could ameliorate erectile dysfunction and structural changes in various types of ED models.

Penile erection is a series of vascular event closely related to the endothelium and smooth muscle cells of corpus cavernosum, which histologically form the basic structure of sinusoids. When the smooth muscle is contracted, the blood inflow through the cavernous artery limitedly, but blood outflows through the subtunical venular plexus freely, thus resulting the flaccid state of penis.[31] Upon sexual stimulation, non-adrenergic non-cholinergic (NANC) nerve fibers release nitric oxide (NO), which activates guanylyl cyclase to increase the concentration of cyclic guanosine monophosphate (cGMP). Besides, acetylcholine released from parasympathetic cholinergic nerve fibers causes activation of adenylyl cyclase, increasing the concentration of cyclic adenosine monophosphate (cAMP). The high level of cGMP and cAMP decrease intracellular Ca2 + levels and lead to smooth muscle cell relaxation, followed by normal erection. If any of these processes is interrupted, erectile dysfunction may happen. For example, cavernous nerves injury causes downregulation in nerve signaling of the corpora cavernosa, which reduces NO level in smooth muscle, increases apoptosis in both smooth muscle and endothelium of blood vessels, and upregulates fibro-genetic cytokines to form collagenization of the smooth muscle. These functional and structural changes lead to Veno-occlusive dysfunction[32–34]. Hypoxia can cause a decrease in prostaglandin E1 levels of corpora cavernosa, which commonly inhibit pro-fibrotic cytokines, such as TGFβ1.[35,36] These pro-fibrotic cytokines enhance collagen deposition, decrease the smooth muscle content, reduce elasticity of the penis, and impair the ability of the cavernosa to compress the subtunical veins, causing veno-occlusive dysfunction[32]. As reported, the mechanisms of diabetic ED observed in rat models may include elevated glycation end-products and oxygen free radical levels, impaired synthesis of nNOS, and decreased cGMP- dependent kinase-1[37,38]. In a word, ED is a multifactorial condition with a complex neurovascular process, which has been shown to be strongly associated with the loss and dysfunction of the corporal endothelium and smooth muscle.

Clinically, refractory male erectile dysfunction shows resistance to drug therapy, which has little effect on them. Facing this obstacle, stem cell therapy was recognized as a promising novel method in ED treatment and considerable studies have proved its feasibility in both animal models and clinical trials, mostly having significant effects[39,40].

Stem cells derived from embryonic or adult tissues, have the capability of self-renewal, proliferation and multipotential differentiation. The regenerative properties have been established in tissue engineering and regenerative medicine researches.[41,42]

Recently, some studies considered that the beneficial effects of transplanted stem cells could not be merely explained by engraftment or differentiation into specific cells.[43] Scientists have paid more attention to the paracrine secretion of stem cells, including chemoattractant molecules, bioactive factors and EVs,[44,45] which play a key role in the paracrine secretion. Exosomes are 50–100 nm membrane-bound EVs, in which content varies dependent on the original cells and the activation status, including non-coding small RNAs, mRNAs, proteins, and lipids[46]. Exosomes have been proved to serve multiple physiologic and pathologic functions via regulating intercellular communication. The therapeutic potential of exosomes in different diseases is a hot topic to date. Lai et al[47]. identified exosomes derived from mesenchymal stem cells (MSCs) exerted protective effect on cardiac tissue following myocardial infarction (MI). Zhang et al[48]. demonstrate that MSCs derived exosomes effectively promoted functional recovery in rats after traumatic brain injury by facilitating endogenous angiogenesis and neurogenesis.

Corresponding to previous stem cell therapy studies, our study revealed that stem cell derived exosomes increased the smooth muscle to collagen ratio, and the expression of α-SMA, CD31, nNOS and eNOS damaged by ED. CD31 can be considered biomarker of endothelium contents[49], while a-SMA and SM/Collagen indicated the smooth muscle contents in the corpus cavernosum of rats. This meant exosomes can improve corpus cavernosum tissue structure to ameliorate erectile function and then the therapeutic efficacy can last for a longer time. Besides, our study proved the downregulation expression level of TGF-β1 and caspase3. As a kind of profibrotic cytokine, TGFβ1was recognized as a key factor related to the formation and development of corporal fibrosis such as in PD.[50] Kim et al reported that the activation of TGF-β1 signaling initiated collagen accumulation and deposition.[51] Activation of caspases was recognized as the biochemical marker for apoptosis and detection of caspase 3 was widely used in apoptotic signals examination research[52]. Vasculogenic ED induced by artery injury was characterized as the ischemic and hypoxic state of corpus cavernosum, which may increase the release of reactive oxygen species (ROS), leading to cell apoptosis[53,54]. It is reported that oxidative stress is an important factor of ED progress for penile ischemia. Liu et al[55] found that cell apoptosis can be induced by ROS, which also increased cytoprotective autophagy, slightly reducing apoptosis. In our study, the administration of stem cell derived exosomes decreased the expression level of caspase 3 and TGF-β1, which indicated that exosomes possess the ability to inhibit fibrosis and apoptosis, ensuring the functional endothelium and smooth muscle contents in corpus cavernosum. Our study also demonstrated that stem cells derived exosomes can make functional changes of corpus cavernosum via the NO/cGMP signaling pathway, [31]which was the most important mechanism known to regulate erection. The eNOS synthesized by endothelial cells and nNOS[56] in cavernous nerves both significantly increased after exosomes therapy in our study. The outcome was consistent with ICP/MAP analysis.

In the included studies, only two studies used exosomes generated from human urine-derived stem cells, [28,29]the others used exosomes derived from ADSCs or BMSCs. They showed no difference to the therapeutic efficacy in our research. Although exosomes can be generated by most cells, the exosomes derived from MSCs were used in most researches to treat ED. MSCs were more widely used than other stem cells in researches for the superiority of abundant tissue source and easy isolation. MSCs can be isolated from several tissues, including bone marrow, adipose tissue, Wharton's Jelly (WJ) tissue, umbilical cord blood, and neonatal teeth.[57] Furthermore, ADSC and BMSC were exploited mostly. Non-coding RNAs, such as miRNA, snoRNA, tRNA, enriched in exosomes, may exert important biological functions, conveying properties of parental cells. For example, tRNAs accounted over 50% proportion of total small RNAs in exosomes derived from adipose-derived stem cells, while represented 23–25% in BMSC derived exosomes. Besides, specific tRNAs were abundant in exosomes compared to the cell. Interestingly, miRNAs were the major content of the cellular small RNA in MSCs, and the discrepancy may suggest preferential sorting and release.[46,58] Exosomes may exhibit heterogeneity of content even originated from the same parental cells.[59–61] Both the subcellular origin and cell activation status were responsible for molecular heterogeneity of exosomes.[62,63] Because of the limitation of exosome isolation methods, bulk isolates rather than pure exosome population isolates were used in majority of studies when evaluating their therapeutic efficacy.[46]

Exosomes isolated from urine also contained substantial non- coding small RNAs, such as tRNA and rRNA, while the exact functions need further study. In fact, the researches on exosome-mediated communication mostly focused on well-known RNA species such as miRNAs and mRNAs, for the challenges on detection sensitivity and specificity of exosomes contents.[46] Zhu et al[25] found that ADSC derived exosomes contained some microRNAs with proangiogenic (miR-126, miR-130a and miR-132) and antifibrotic (miR-let7b and miR-let7c) functions. Besides the membrane proteins of exosomes influencing the interaction with recipient cells, there were functional proteins intra exosomes involving intracellular signaling mediation. Wang et al[23] used the transmembrane serine protease Corin in ADSC derived exosomes to improve erectile dysfunction in diabetic rats and suggested that it may play a role through the ANP/NO/cGMP signaling pathway.

Compared with stem cell therapy, exosomes have many advantages, including (i) greater stability and ease of storage and management, (ii) preclusion the risk of tumor formation, and (iii) a lower likelihood of an immune rejection.[14,21]

IC injection was the main method to administrate cells or exosomes into the cavernous body in most researches. However, in terms of histologically high vascularization, effective retention of exosome or stem cells in local tissue is a key challenge. Exosomes derived from mature body cells may have the superiority of better histocompatibility, compared with that derived from stem cells. Song et al[27] thought that exosomes generated from corpus cavernosum smooth muscle cells (CCSMCs) were more easily taken up and retained in the corpus cavernosum of diabetic ED rats than those from BMSC or ADSC.

In this study, we analyzed the efficiency of exosome therapy for ED using a meta-analysis to obtain a powerful conclusion. To the best of our knowledge, this is the first meta-analysis providing comprehensive insights into the effects of stem cell derived exosomes on ED in rats. The value of systematic review of experimental animal studies has been steadily understood.[64,65] The consistent results of exosome therapy efficacy across various ED models in our study would provide reassurance that human beings might respond in the same way. While, here are still several limitations in this study. High degree of heterogeneity remains in ICP/MAP outcome after subgroup analysis. This may be due to the methodological heterogeneity of the included studies, as exosome types, extraction methods and animal models used in each study were quite different. In view of the limited number of included studies, more

accurate subgroup analysis cannot be performed at present. Besides, different software (e. g. SPSS, GraphPad Prism and Stata) are applied in included studies, which may cause high statistical heterogeneity.

The egger test shows publication bias in our study, which may affect the credibility of the conclusions. However, we have tried our best to retrieve animal intervention studies on exosome treatment for ED, including preprint database like bioRxiv and medRxiv, but failing to find more relevant researches. This indicates that exosome therapy for ED is a relatively new topic, and more experimental data are needed to support its effectiveness.

However, in the meta-analysis of structural changes, low heterogeneity of SM/ Collagen, CD31, and eNOS outcomes guaranteed the credibility of the conclusion that exosome treatment ameliorated the cavernosum structure, and verified the ICP/MAP functional recovery from the side.

Conclusion

The study suggests the efficacy of stem cell derived exosome therapy for various ED models. IC injection of exosomes ameliorate the cavernosum structure by increasing muscle content and decreasing collagen to improve erectile function. Stem cell derived exosomes have great potential to afford a novel cell- free therapy for ED treatment compared with stem cell therapy, while further clinical trials are needed to demonstrate the actual effects on human body.

Abbreviations

ED: Erectile dysfunction; ICP/MAP: Intracavernosal pressure/mean artery pressure; SM/Collagen: The ratio of smooth muscle to collagen; α-SMA: Alpha smooth muscle actin; CD31: The cluster of differentiation 31; nNOS: Neuronal nitric oxide synthase; eNOS: Endothelial nitric oxide synthase; TGF-β1: Transforming growth factor-β1; MSC: Mesenchymal stem cell; BMSC: Bone marrow-derived mesenchymal stem cell; ADSC: Adipose-derived mesenchymal stem cell; OSA: Obstructive sleep apnea; PD: Peyronie's disease; MD: Mean difference; CI: Confidence intervals; cGMP: Cyclic guanosine monophosphate; cAMP: cyclic adenosine monophosphate; EVs: extracellular vesicles; IC: Intra-cavernous.

Declaration

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Figures

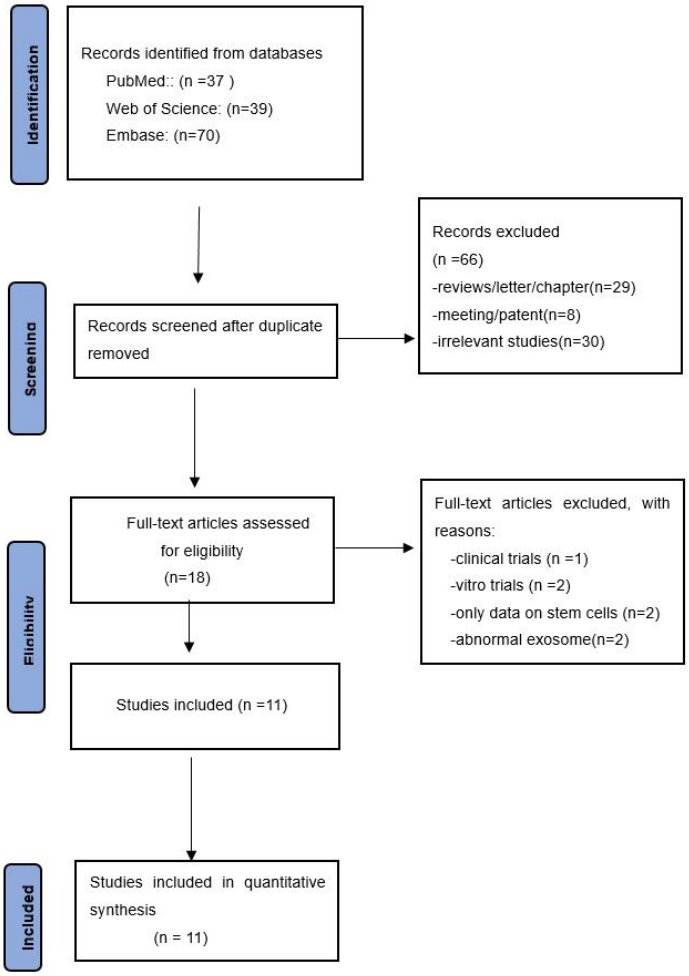
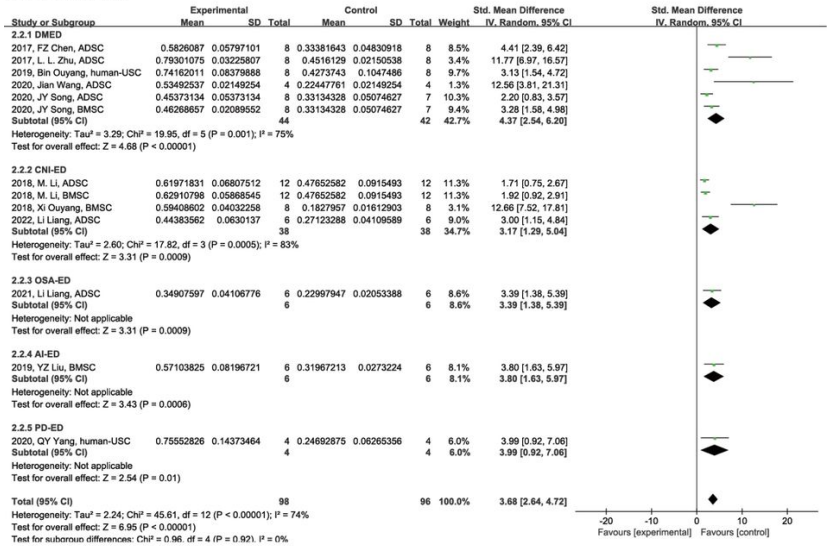


Figure 1

Flowchart of study selection.

A. Producer cell



B. ED model type

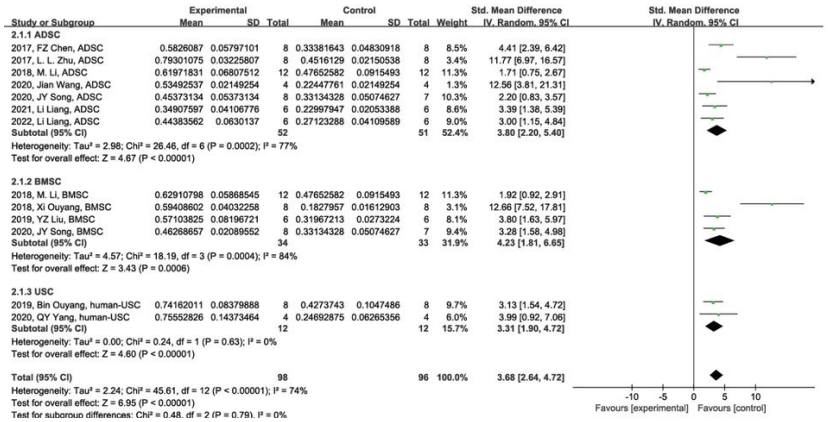
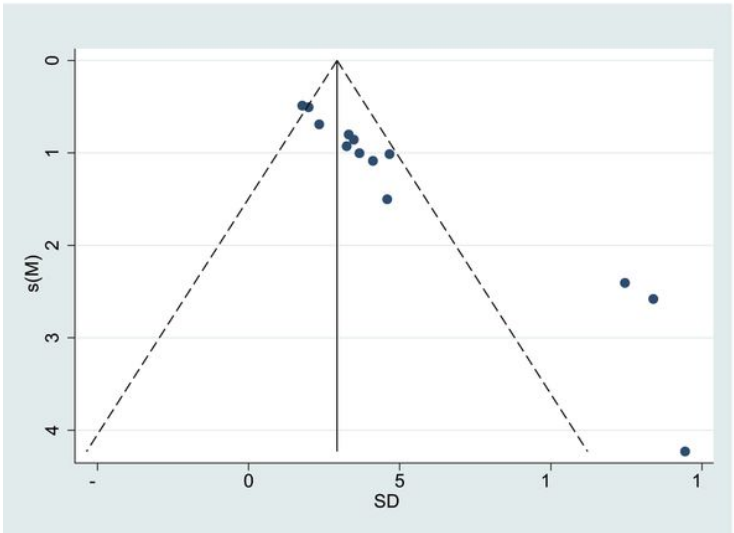


Figure 2

The comparison of ICP/MAP changes among different subgroups. (A) producer cell, (B) ED model type

A. Funnel plot



B. Egger's publication bias plot

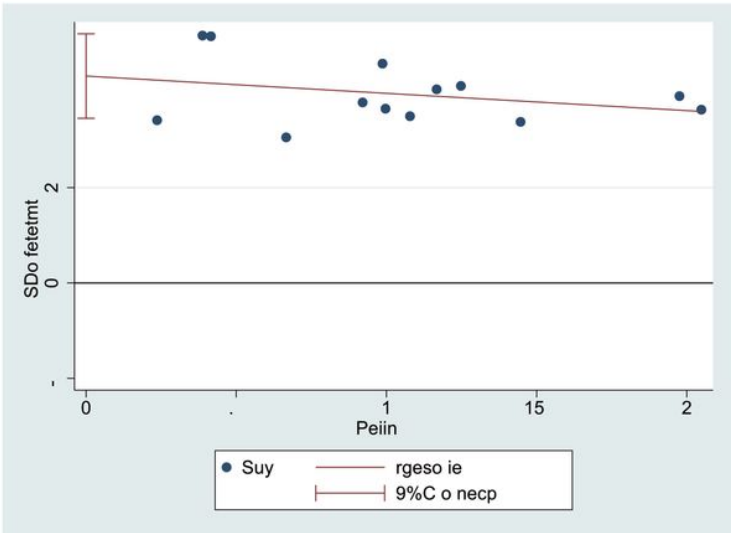


Figure 3

Publication bias test of ICP/MAP. (A) Funnel plot, (B) Egger's publication bias plot