Supplementary File S1.

Systematic Review Protocol for Animal Intervention Studies

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Version 2.0 (December 2014)

Item #	Section/Subsection/Item	Description	Check for approval
A. General			
1.	Title of the review	Adipose tissue-derived stem cell therapy for cavernous nerve injury-induced erectile dysfunction in the rat model: A systematic review and meta-analysis	X
2.	Authors (names, affiliations, contributions)	Hyo Jung Park-Biomedicine&Health sciences, The Catholic University, Repulic of Korea-study concept and design, eligibility screening, data extraction, protocol preparation, data analysis, risk of bias assessment, manuscript writing Hyunsuk Jeong-Preventive medicine, The Catholic University, Repulic of Korea- study design, scientific concept, protocol preparation, eligibility screening, data extraction, data analysis and interpretation, risk of bias assessment, critical review of the manuscript Ji Youl Lee-Urology, Biomedicine&Health sciences, The Catholic University, Republic of Korea-study design, scientific concept, protocol preparation, eligibility screening, data extraction, data analysis and interpretation, risk of bias assessment, critical review of the manuscript Na Jin Kim-Medical Library, The catholic University of Korea, Seoul, Republic of Korea- literature search strategies, data search and validation Yong Hyung Park- Department of Urology, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea- study design, data verification, critical review of the manuscript U-syn Ha- Department of Urology, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea- comment on protocol, critical review of the manuscript Sung-Hoo Hong- Department of Urology, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea- comment on protocol, critical review of the manuscript Sae Woong Kim- Department of Urology, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea-comment on protocol, critical review of the manuscript	X
3.	Other contributors (names, affiliations, contributions)		
4.	Contact person+e-mail address	Ji Youl Lee, uroljy@catholic.ac.kr Hyunsuk Jeong, suejeong@catholic.ac.kr	X
5.	Funding sources/sponsors	None	
6.	Conflicts of interest	No conflicts of interests	X
7.	Date and location of protocol registration	NA	
8.	Registration number (if applicable)	NA	
9.	Stage of review at time of registration	NA	

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B. Objectives			
Background			
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	Prostate cancer is the second most frequent cancer and the fifth leading cause of cancer death in men worldwide The majority of prostate cancer, about 80%, is identified as clinically localized and treated with radical prostatectomy. Despite its surgical technique advance-ments, many patients experience erectile dysfunction after prostatec-tomy because of cavernous nerve injury. Type-5 phospho-diesterase inhibitors are the first line drugs for ED, but PDE5Is provide only symptomatic relief of ED and do not offer a cure for the disease. Therefore there is a growing interest in developing therapies including stem cell therapy that offer a cure for the disease. Adipose tissue-derived stem cells (ADSCs) are a mesen-chymal stem cell source that can easily be isolated from adipose tissue. From now on, a couple of meta-analyses have been reported about efficacy of various type of stem cell therapy or ED rat model. They reported the efficacy of stem cell therapy on ED rat model but they did not evaluate the efficacy of stem cell with consideration for methodological quality assessment. In the clinical study, randomization controlled trial is the rigorous study design to evaluate the efficacy of intervention. However the background of animals was basically homogeneous, most of the animal studies didn't mention the procedure of randomization. It might be not necessary for animals to be blinded because they don't have any desire for the treatment. But blinding of outcome assessor is important in animal studies, because researchers may anticipate positive results in their experimental studies As in human RCTs, animal studies also should have high quality of study design and analysis to ensure the efficacy of intervention which is not affected by conscious or unconscious bias. Therefore, we analyzed the effect of ADSCs on ED induced by CNI in rats. Furthermore we explored how proper blinding of outcome assessor affected outcomes in animal studies.	X
Research question			
11.	Specify the disease/health problem of interest	Erectile dysfunction due to cavernous nerve injury	X
12.	Specify the population/species studied	Rats	X
13.	Specify the intervention/exposure	ADSCs	X
14.	Specify the control population	None	X
15.	Specify the outcome measures	ICP/MAP, nNOS, smooth muscle/collagen ratio, cGMP	X
16.	State your research question (based on items 11～15)	What is the effect of adipose derived stem cell on erectile dysfunction in experimental cavernous nerve injury?	X

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C. Methods			
Search and study identification			
17.	Identify literature databases to search (e.g. Pubmed, Embase, Web of science)	**X** MEDLINE via PubMed **X** Web of Science ☐ SCOPUS **X** EMBASE **X** Other, namely: Cochrane ☐ Specific journal(s), namely:	X
18.	Define electronic search strategies (e.g. use the step by step search guide (15) and animal search filters (20, 21)	See supplementary file 2	X
19.	Identify other sources for study identification	**X** Reference lists of included studies ☐ Books **X** Reference lists of relevant reviews ☐ Conference proceedings, namely: ☐ Contacting authors/ organisations, namely: ☐ Other, namely:	X
20.	Define search strategy for these other sources	Screening the reference lists for relevant titles and screening the abstracts of these relevant titles	X
Study selection			
21.	Define screening phases (e.g. pre-screening based on title/abstract, full text screening, both)	1) screening based on title and abstract 2) full text screening of the eligible articles	X
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	Each phase: 2 independent observers per article. One observer will screen all articles, HS and HJ will each screen half of the articles. Differences will be solved through discussion or by consulting a fourth investigator.	X
	Define all inclusion and exclusion criteria based on:		
23.	Type of study (design)	Inclusion criteria:studies including a control group undergoing no treatment versus a ADSDc treated group Exclusion criteria: studies without a suitable control group	X
24.	Type of animals/population (e.g. age, gender, disease model)	Inclusion criteria: erectile dysfunction induced by CNI rat model Exclusion criteria: erectile dysfunction rat model induced by other causes	X
25.	Type of intervention (e.g. dosage, timing, frequency)	Inclusion criteria: treatment with ADSCs in any origin of cell, cell number, route of cell injection, follow up periods Exclusion criteria:None	X
26.	Outcome measures	Inclusion criteria:erectile function outcome including ICP/MAP ratio, nNOS, CSM/collagen, cGMP Exclusion criteria:No ICP/MAP ratio reported	X
27.	Language restrictions	Inclusion criteria: English Exclusion criteria: non English	X
28.	Publication date restrictions	Inclusion criteria: all publication date Exclusion criteria: none	X
29.	Other	Inclusion criteria: none Exclusion criteria: none	X

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30.	Sort and prioritize your exclusion criteria per selection phase	Selection phase: title and abstract screening 1. Not in vivo (e.g. ex vivo/in vitro/in sillico) 2. Data published in duplicate 3. Human 4. No CNI rat model Selection phase: full text screening 1. No comparison ADSC versus no ADSC 2. No relevant outcome measure 3. Review 4. No full paper(abstract, comment) 5. No relevant outcome measure	X
Study characteristics to be extracted (for assessment of external validity, reporting quality)			
31.	Study ID (e.g. authors, year)	First author, title, year of publication	X
32.	Study design characteristics (e.g. experimental groups, number of animals)	experimental groups, control groups, number of rats per group	X
33.	Animal model characteristics (e.g. species, gender, disease induction)	Rat species, age	X
34.	Intervention characteristics (e.g. intervention, timing, duration)	Type of control intervention, ADSC origin of cell ADSC cell number, modification, route of cell injection, follow up periods	X
35.	Outcome measures	Primary outcome: ICP/MAP ratio Secondary outcome: nNOS, smooth muscle contents, collagen contents, smooth muscle/collagen ratio, cGMP,	X
36.	Other (e.g. drop-outs)	None	
Assessment risk of bias (internal validity) or study quality			
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	Two reviewers will assess the risk of bias and study quality of all studies reporting on one of the outcome measures selected for meta-analysis Discrepancies will be resolved by discussion.	X
38.	Define criteria to assess (a) the internal validity of included studies (e.g. selection, performance, detection and attrition bias) and/or (b) other study quality measures (e.g. reporting quality, power)	**X** By use of SYRCLE's Risk of Bias tool (4) ☐ By use of SYRCLE's Risk of Bias tool, adapted as follows: ☐ By use of CAMARADES' study quality checklist, e.g (22) ☐ By use of CAMARADES' study quality checklist, adapted as follows: ☐ Other criteria, namely:	X
Collection of outcome data			
39.	For each outcome measure, define the type of data to be extracted (e.g. continuous/dichotomous, unit of measurement)	ICP/MAP ratio, continuous nNOS, continuous CSM/collagen ratio, continuous cGMP continuous in pmol/g	X
40.	Methods for data extraction/retrieval (e.g. first extraction from graphs using a digital screen ruler, then contacting authors)	1. study characteristics using a structured data extraction form 2. direct extraction of data from tables, texts and figures 3. numerical data from graph using a WebPlotDigitizer version 3.8 4. Contacting authors by e-mail for raw data if data not reported or unclear If studies evaluated with multiple treatment groups next to the control groups, they were considered as separate experiments. All outcomes data were extracted mean, standard deviation (SD) and number of animals in both intervention and control groups. If some studies expressed outcome data with the standard error (SE), we changed the data from SE to SD.	X

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41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	One reviewer will extract the data, a second reviewer will check the extracted data for inconsistencies.	X
Data analysis/synthesis			
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	Meta-analysis will be performed for all selected outcomes reported in all of the included studies.	X
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	Meta-analysis will be performed for all selected outcomes reported in all of the included studies.	X
	If a meta-analysis seems feasible/sensible, specify (for each outcome measure):		
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	Differences between the intervention and control groups were expressed as standardized mean differences with 95% confidence intervals (CIs) for continuous variables.	X
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random effects model for all outcome measures	X
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	The heterogeneity was analyzed using I2 statistics and defined as low (25% to 50%), moderate (50%～75%), or high (>75%).	X
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	To explore the source of heterogeneity, we conducted preplanned subgroup analysis on origin of blinding of the outcome assessment (blinded, unblinded), ADSC (autologous, allogenic, human), and follow-up period (<6 weeks, ≥6 weeks).	X
48.	Any sensitivity analyses you propose to perform	NA	X
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	None	X
50.	The method for assessment of publication bias	Publication bias was examined graphically with a contour enhanced funnel plot. If publication bias was suspected, we adjusted the estimate using the trim and fill method.	X

Supplementary File S2.

	Systematic searches (performed on 28 Jan 2019)
PubMed	
CNI	"cavernous nerve injury"[TW] OR CNI[TW] OR "CN injury"[TW] OR "Prostatectomy"[MeSH] OR "Prostatectomy"[TW] OR "Prostatectomies"[TW] OR "Prostatectomy, Suprapubic"[TW] OR "Prostatectomies, Suprapubic"[TW] OR "Suprapubic Prostatectomies"[TW] OR "Suprapubic Prostatectomy"[TW] OR "Prostatectomy, Retropubic"[TW] OR "Prostatectomies, Retropubic"[TW] OR "Retropubic Prostatectomies"[TW] OR "Retropubic Prostatectomy"[TW]
Erectile dysfunction	"Erectile Dysfunction"[MeSH] OR "Erectile Dysfunction"[TW] OR "Dysfunction, Erectile"[TW] OR "Male Sexual Impotence"[TW] OR "Impotence, Male Sexual"[TW] OR "Sexual Impotence, Male"[TW] OR "Male Impotence"[TW] OR "Impotence, Male"[TW] OR "Impotence"[TW] OR ED[TW]
ADSC	"adipose-derived stem cell"[TW] OR "adipose-derived stem cells"[TW] OR "ADSC"[TW] OR "ADSCs"[TW] OR "adipose tissue-derived stem cell"[TW] OR "adipose tissue-derived stem cells"[TW]
EMBASE	
CNI	cavernous nerve injury' OR cni OR 'cn injury' OR 'prostatectomy'/exp OR 'prostatectomy' OR 'prostatectomies' OR 'prostatectomy, suprapubic' OR 'prostatectomies, suprapubic' OR 'suprapubic prostatectomies' OR 'suprapubic prostatectomy' OR 'prostatectomy, retropubic' OR 'prostatectomies, retropubic' OR 'retropubic prostatectomies' OR 'retropubic prostatectomy'
Erectile dysfunction	'erectile dysfunction'/exp OR 'erectile dysfunction' OR 'dysfunction, erectile' OR 'male sexual impotence' OR 'impotence, male sexual' OR 'sexual impotence, male' OR 'male impotence' OR 'impotence, male' OR 'impotence' OR ed
ADS	OR 'adipose derived stem cell'/exp OR 'adipose-derived stem cell' OR 'adipose-derived stem cells' OR 'adsc' OR 'adscs' OR 'adipose tissue-derived stem cell' OR 'adipose tissue-derived stem cells'
Cochrane	
CNI	"cavernous nerve injury":ti,ab,kw OR CNI:ti,ab,kw OR "CN injury":ti,ab,kw OR [mh "Prostatectomy"] OR "Prostatectomy":ti,ab,kw OR "Prostatectomies":ti,ab,kw OR "Prostatectomy, Suprapubic":ti,ab,kw OR "Prostatectomies, Suprapubic":ti,ab,kw OR "Suprapubic Prostatectomies":ti,ab,kw OR "Suprapubic Prostatectomy":ti,ab,kw OR "Prostatectomy, Retropubic":ti,ab,kw OR "Prostatectomies, Retropubic":ti,ab,kw OR "Retropubic Prostatectomies":ti,ab,kw OR "Retropubic Prostatectomy":ti,ab,kw
Erectile dysfunction	OR [mh "Erectile Dysfunction"] OR "Erectile Dysfunction":ti,ab,kw OR "Dysfunction, Erectile":ti,ab,kw OR "Male Sexual Impotence":ti,ab,kw OR "Impotence, Male Sexual":ti,ab,kw OR "Sexual Impotence, Male":ti,ab,kw OR "Male Impotence":ti,ab,kw OR "Impotence, Male":ti,ab,kw OR "Impotence":ti,ab,kw OR ED:ti,ab,kw
ADSC	OR "adipose-derived stem cell":ti,ab,kw OR "adipose-derived stem cells":ti,ab,kw OR "ADSC":ti,ab,kw OR "ADSCs":ti,ab,kw OR "adipose tissue-derived stem cell":ti,ab,kw OR "adipose tissue-derived stem cells":ti,ab,kw
Web of Science	
CNI	TS=("cavernous nerve injury" OR CNI OR "CN injury") TS=("Prostatectomy" OR "Prostatectomies" OR "Prostatectomy, Suprapubic" OR "Prostatectomies, Suprapubic" OR "Suprapubic Prostatectomies" OR "Suprapubic Prostatectomy" OR "Prostatectomy, Retropubic" OR "Prostatectomies, Retropubic" OR "Retropubic Prostatectomies" OR "Retropubic Prostatectomy")
Erectile dysfunction	TS=("Erectile Dysfunction" OR "Dysfunction, Erectile" OR "Male Sexual Impotence" OR "Impotence, Male Sexual" OR "Sexual Impotence, Male" OR "Male Impotence" OR "Impotence, Male" OR "Impotence" OR ED)
ADSC	**TS=("adipose-derived stem cell" OR "adipose-derived stem cells" OR "ADSC" OR "ADSCs" OR "adipose tissue-derived stem cell" OR "adipose tissue-derived stem cells")**