SYSTEMATIC REVIEW PROTOCOL FOR ANIMAL INTERVENTION STUDIES

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Item #	Section/Subsection/Item	Description	Check for approval
	A. General		
1.	Title of the review	L-Arginine during pregnancy – a preclinical meta-analysis on fetal growth and maternal blood pressure	X
2.	Authors (names, affiliations, contributions)	Fieke Terstappen*[1], Nina D. Paauw*[1], Wessel Ganzevoort[2], Kim E. Wever[3], Jaap A. Joles[4], Hendrik Gremmels[4], A. Titia Lely[1] * Authors contributed equally to the work. [1] Dept. of Obstetrics, Wilhelmina Children's Hospital, the Netherlands [2] Dept. of Obstetrics, Academic Medical Center, Amsterdam, the Netherlands [3] SYRCLE, Nijmegen Institute for Health Sciences, Radboud University Medical Center, Nijmegen, Netherlands. [4] Dept. of Nephrology and Hypertension, University Medical Center Utrecht, the Netherlands	X
3.	Other contributors (names, affiliations, contributions)	N/A	X
4.	Contact person + e-mail address	F. Terstappen Division Woman and Baby University Medical Center Utrecht Postbus 85090, 3508 AB Utrecht, The Netherlands Tel: +31 88 755 7526/ Fax: +31 88 755 5436 E-mail: F.Terstappen@umcutrecht.nl	X
5.	Funding sources/sponsors	This study was supported by ZonMw MKMD Synthesis of Evidence (114024115), the Dutch Kidney Foundation (15O141) and ZonMw Clinical Fellowship (40-000703-97-12463).	X
6.	Conflicts of interest	None	X
7.	Date and location of protocol registration	www.syrcle.nl, January 11th 2018	
8.	Registration number (if applicable)	N/A	
9.	Stage of review at time of registration	Preliminary searches completed	
10.	What is already known about this disease/model/intervention? Why is it important to do this review?	L-Arginine is an amino acid that is available as supplement. L-Arginine is involved in several pathways, among others L-Arginine is a precursor of nitric oxide. Nitric oxide might play an important role in vascular health of the placenta. Currently, multiple clinical trials have been performed to study the effect of L-Arginine on fetal growth restriction (FGR) and preeclampsia (PE). However, no clear conclusion has been drawn, mostly due to limitation in study design (small sample size, low dose, low risk groups). Before	X

		starting new human trial, we aim to combine both human and animal trials to identify modifiable factors contributing to the efficacy of L-Arginine. Important factors might be dose, timing or characteristics of population.	
11.	Specify the disease/health problem of interest	Pregnancy complications, such as fetal growth restriction (FGR), preeclampsia (PE), pregnancy induced hypertension (PIH) or risk of FGR/PE.	X
12.	Specify the population/species studied	All pregnant mammals (including humans). The data will be analysed separately for healthy pregnancy and complicated pregnancies.	X
13.	Specify the intervention/exposure	(chronic) L-Arginine supplementation during pregnancy	X
14.	Specify the control population	The control group is the untreated group for both the complicated or healthy pregnancies (no growth restriction or preeclampsia).	X
15.	Specify the outcome measures	1) Fetal or birth weight 2) Maternal blood pressure 3) Development of preeclampsia (PE) or small for gestation age (SGA)	X
16.	State your research question (based on items 11-15)	Does L-arginine supplementation during pregnancy improve fetal growth or maternal blood pressure or can it prevent the development of PE or SGA. Sub-question: which factors in study design influence the efficacy of L-Arginine to treat or prevent preeclampsia or fetal growth restriction? (important factors might be dose, timing or characteristics of population)	X
17.	Identify literature databases to search (*e.g.* Pubmed, Embase, Web of science)	☒MEDLINE via PubMed ☐Web of Science ☐SCOPUS ☒EMBASE ☐Other, namely: ☒Specific journal(s), namely: Cochrane Library	X
18.	Define electronic search strategies (*e.g.* use the step by step search guide[15] and animal search filters[20, 21])	When available, please add a supplementary file containing your search strategy: [Tabel search string]	X
19.	Identify other sources for study identification	☒Reference lists of included studies ☐Books ☒Reference lists of relevant reviews ☐Conference proceedings, namely: ☐Contacting authors/ organisations, namely: ☐Other, namely:	X
20.	Define search strategy for these other sources	Examination of reference lists of included studies or relevant reviews and determine if references have been identified through search terms; include for evaluation if it meets the same criteria.	X

21.	Define screening phases (*e.g.* pre-screening based on title/abstract, full text screening, both)	Both: 1) Title/abstract screening 2) Full text screening	X
22.	Specify (a) the number of reviewers per screening phase and (b) how discrepancies will be resolved	2 independent researchers (N.D.P. and F.T.) will screen literature for both screening phases 1) and 2). Discrepancies will be discussed between the two researchers until consensus is reached, decision by a third investigator (H.G.) if no consensus is reached.	X
23.	Type of study (design)	Inclusion criteria: all animal studies and RCT studies in case of human trials Exclusion criteria: other study design than RCT studies in human trials, experimental animal case reports	X
24.	Type of animals/population (*e.g.* age, gender, disease model)	Inclusion criteria: pregnancy (healthy, complicated, high risk population); no criteria on age Exclusion criteria: non-pregnant mammals, pregnant non-mammals, lysinuric protein insufficiency/intolerance, no separate control group	X
25.	Type of intervention (*e.g.* dosage, timing, frequency)	Inclusion criteria: L-Arginine supplementation during pregnancy, for at least more than one day or single dose Exclusion criteria: Treatment less than one day or single dose, combined intervention, continuation of treatment after birth	X
26.	Outcome measures	Inclusion criteria: fetal or birth weight, maternal blood pressure after treatment, development of PE/SGA Exclusion criteria: blood pressure measurement too close to start of supplementation of L-Arginine (<1 day).	X
27.	Language restrictions	Inclusion criteria: All Exclusion criteria: No language restriction	X
28.	Publication date restrictions	Inclusion criteria: all Exclusion criteria: none	X
29.	Other	Inclusion criteria: N/A Exclusion criteria: no untreated control group	X
30.	Sort and prioritize your exclusion criteria per selection phase	Title/abstract: - No L-arginine or no separate control group - No other study design than RCT in human trials or experimental animal case report - Type of animal/population Full-text: - Exclusion of studies with co-intervention - Treatment less than one day/single dose - Measurement too close to start of supplementation	X
31.	Study ID (*e.g.* authors, year)	1) First author 2) Year of publication 3) PMID	X
32.	Study design characteristics (*e.g.* experimental groups, number of animals)	1) Experimental groups 2) Number of humans/animals per group 3) Human: inclusion criteria	X

33.	Animal model characteristics (*e.g.* species, gender, disease induction)	1) Species 2) Model 3) Strain 4) Pregnancy type (FGR, PE or healthy) 5) Breeding 6) Body weight in grams 7) Baseline protein intake	X
34.	Intervention characteristics (*e.g.* intervention, timing, duration)	1) Dose in g/kg body weight/day 2) Administration scheme (continuous vs. interval) 3) Administration timing and category (early, middle, late, full gestational) 4) Administration route (only for sub-analysis) 5) Type of intervention (treatment vs. prevention)	X
35.	Outcome measures	1) Fetal or birth weight - Mean and SD or SEM - N dams - N pups - Sex of offspring - Gestational age at measurement 2) Maternal blood pressure - Mean and SD or SEM - N dams - Gestational age at measurement - Type of blood pressure measurement 3) Development of PE/SGA - Yes/no - N cases	X
36.	Other (*e.g.* drop-outs)	N/A	X
37.	Specify (a) the number of reviewers assessing the risk of bias/study quality in each study and (b) how discrepancies will be resolved	(a) Two independent researchers (N.D.P and F.T.) (b) By discussion between the two researchers until consensus is reached, decision by a third investigator (H.G.) if no consensus is reached.	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)	☒ 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: Cochrane Risk of Bias tool for human studies	X
39.	For each outcome measure, define the type of data to be extracted (*e.g.* continuous/dichotomous, unit of measurement)	1) Fetal or birth weight - Continuous; grams 2) Maternal blood pressure - Continuous; mmHg 3) Development of PE or SGA - PE defined as combination of blood pressure > 140/90 mmHg and proteinuria; dichotomous - SGA defined as birth weight <p10 or 2500	X

		gram; dichotomous	
40.	Methods for data extraction/retrieval (*e.g.* first extraction from graphs using a digital screen ruler, then contacting authors)	1) In text or tables 2) Extracted from graphs using graph digitizers 3) Contacting authors per email (one attempt) 4) Missing data: conservative estimate	
41.	Specify (a) the number of reviewers extracting data and (b) how discrepancies will be resolved	2 independent researchers (N.D.P. and F.T.) will extract data). Discrepancies will be discussed between the two researchers until consensus is reached, decision made by a third investigator (H.G.) if no consensus is reached.	X
42.	Specify (per outcome measure) how you are planning to combine/compare the data (*e.g.* descriptive summary, meta-analysis)	1) Fetal or birth weight: in grams - Compared as ratio of means - In case of multiple time points within one study a correction will be performed 2) Maternal blood pressure: SBP, DBP, MAP in mmHg - Compared as mean difference - In case of multiple time points within one study a correction will be performed 3) Development of PE or SGA (% yes/no) in high risk population Outcomes will be analysed separately for FGR/PE, risk population and healthy pregnancies. For the meta-analysis only studies with oral administration will be included.	X
43.	Specify (per outcome measure) how it will be decided whether a meta-analysis will be performed	Decision whether to perform meta-analysis is based on number of studies. Overall: minimal 5 studies; For subanalysis minimal 2 per category	X
44.	The effect measure to be used (*e.g.* mean difference, standardized mean difference, risk ratio, odds ratio)	1) Fetal or birth weight - Compared as ratio of means 2) Maternal blood pressure - Compared as mean difference 3) Development of PE/SGA - Risk ratio	X
45.	The statistical model of analysis (*e.g.* random or fixed effects model)	Random and mixed effects model	X
46.	The statistical methods to assess heterogeneity (*e.g.* I^2, Q)	$I^2 > 50\%$ will be considered significant	X
47.	Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis)	Sub-analysis will only be performed in complicated pregnancies: 1) Fetal or birth weight - Species - Administration timing (early, mid, late or full gestation) - Administration scheme (continuous vs. interval) - Administration route - Prevention or treatment - Percentage of growth restriction - Protein intake 2) Maternal blood pressure - Species	X

		- Administration timing (early, mid, late or full gestation) - Administration scheme (continuous vs. interval) - Administration route - Prevention or treatment - Base-line blood pressure - Protein intake In risk population: 3) Development of PE/SGA - Species - Administration timing (early, mid, late or full gestation) - Administration scheme (continuous vs. interval) - Administration route - Protein intake	
48.	Any sensitivity analyses you propose to perform	Influential case analysis will be performed by examining residuals, weights and Cook's distances of model fits. Sensitivity analysis will be performed by removing influential cases and by shifting cut-out for subgroup analysis of administration timing.	X
49.	Other details meta-analysis (*e.g.* correction for multiple testing, correction for multiple use of control group)	- Multilevel models will be used to account for within-study clustering of data points in case of multiple doses used in one study - Multilevel model correction for multiple use of control groups - Post-hoc multiple testing correction	X
50.	The method for assessment of publication bias	In case of >20 studies: contour-enhanced funnel-plots and Egger's regression test in case of funnel plot asymmetry.	X

Final approval by (names, affiliations):

F. Terstappen, MD, MSc
N.D. Paauw, MD, MSc
W. Ganzevoort, MD, PhD
K.E. Wever, PhD
J.A. Joles, DVM, PhD
H. Gremmels, MD, PhD
A.T. Lely, MD, PhD

Date:

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