

# The Association Between the Risk of Hypertensive Disorders of Pregnancy and Folic Acid: A Systematic Review and Meta-Analysis

Yahui Yu<sup>1,2,3</sup>, Ximu Sun<sup>1,2,3</sup>, Xinrui Wang<sup>1,2,3</sup>, and Xin Feng<sup>1,2</sup>

<sup>1</sup>Department of Pharmacy, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, Beijing, China; <sup>2</sup>Department of Pharmacy, Beijing Maternal and Child Health Care Hospital, Beijing, China; <sup>3</sup>Department of Clinical Pharmacology, College of Pharmaceutical Sciences, Capital Medical University, Beijing, China

**Corresponding Author:** Xin Feng, Department of Pharmacy, Beijing Obstetrics and Gynecology Hospital, Capital Medical University, No.17, Qi He Lou Street, Dongcheng District, Beijing, China; TEL: (+86) 10 52275218; Fax: (+86) 10 52275218; email: [fengxin1115@126.com](mailto:fengxin1115@126.com)

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**ABSTRACT -- Purpose:** Although folic acid (FA) supplementation has been shown to reduce general cardiovascular risks, its impact on hypertensive disorders of pregnancy (HDP) is unclear. We performed a systematic review and meta-analysis to clarify the association between FA and the risk of HDP (pre-eclampsia (PE) and gestational hypertension (GH)). **Methods:** PubMed, EmBase, and Cochrane Library were searched up to June 18, 2020, stratified by type of disease, initiation time of FA, form of FA and pre-conception Body Mass Index (BMI). The quality assessment of included studies was evaluated using Newcastle-Ottawa Scale (NOS) for cohort studies and Cochrane Collaboration's Risk of Bias Assessment Tool for randomized controlled trials (RCTs). Between-study heterogeneity was quantified using Cochran's Q-statistic and  $I^2$  statistics. Sensitivity analysis was performed by excluding the studies one by one, and publication bias was analyzed using funnel plots. **Results:** Twenty studies with 359041 patients were identified for inclusion in the meta-analysis which included 3 RCTs and 17 cohort studies. Pooled estimates showed RR of 0.83 (95% CI 0.74-0.93,  $P=0.0008$ ) for association between low dose FA (LD-FA) and the risk of PE, but LD-FA was not associated with GH (RR 1.05, 95% CI 0.97-1.13,  $P=0.20$ ). In addition, the results of subgroup analysis showed that post-conception LD-FA had a 31% decreased risk of PE (RR 0.69, 95% CI 0.59-0.80,  $P<0.00001$ ), and LD-FA in patients with pre-conception BMI $<25$  kg/m<sup>2</sup> had a 32% decreased risk of PE (RR 0.68, 95% CI 0.56-0.81,  $P<0.0001$ ) **Conclusions:** LD-FA significantly decreased the risk of PE but not GH, and post-conception LD-FA and pre-conception BMI $<25$  kg/m<sup>2</sup> were considered as protective factors to reduce the risk of PE.

## INTRODUCTION

Hypertensive disorders of pregnancy (HDP) accounts for 16% of maternal deaths and is also associated with severe morbidity, long term disability and death among both mothers and their babies (1). This group of diseases includes pre-eclampsia (PE) and eclampsia, gestational hypertension (GH) and chronic hypertension. In addition, some evidence showed that women with HDP were at increased risk of developing chronic kidney disease, cardiovascular disease (CVD) and even cardiovascular mortality (2-3). Therefore, effective prevention and treatment of HDP is critical. At present, several clinical practice guidelines have clearly recommended that aspirin could be used for the prevention of HDP in high-risk pregnant women (1-3). A meta-analysis which with focus on the general cardiovascular health indicated a 10% lower

risk of stroke and a 4% lower risk of overall CVD with folic acid (FA) supplementation (4). However, studies investigating the use of FA to prevent HDP have yielded inconsistent results. Currently, the relationship between FA and HDP has been widely studied (5-27), and five meta-analyses with inconsistent results have been published (28-32). Two meta-analyses demonstrated that FA was not associated with the risk of GH or PE (28-29). Two demonstrated that multivitamin containing FA (Vit-FA) could significantly lower GH or PE risk (30-31). And one showed no significant difference between FA alone and Vit-FA in reducing PE (32). The latest retrieval deadline for these meta-analyses was Aug. 2017. Since then, six other studies have been published. In addition, FA initiation time, and pre-conception Body Mass Index (BMI) were used as grouping conditions in some cohort studies, so these

factors are an important opportunity for subgroup analysis of meta-analysis.

The purpose of this study was to perform a systematic review and meta-analysis to clarify the association between FA and the risk of HDP.

## MATERIALS AND METHODS

### Search Strategy

We conducted a comprehensive search in PubMed, EmBase, and Cochrane Library from the date of their inception up to June 18, 2020. The search terms were composed of the following: “folic acid”, “vitamin b9”, “preeclampsia”, “pregnancy induced hypertension”, “gestational hypertension”, “hypertensive disorders of pregnancy”. To identify potential publications, we also examined the references of relevant reviews and meta-analysis. The details of the search strategy were summarized in the Appendix 1.

### Study Selection

Inclusion criteria: (1) population-based studies, including cohort studies, case-control studies, and randomized controlled trials (RCTs). (2) the exposure factor is FA or Vit-FA. (3) the outcome is GH or PE. (4) the written language is English.

Exclusion criteria: (1) not original studies (e.g., reviews, meta-analysis, commentaries, case reports, editorials and letters). (2) conducted in animals. (3) studies without control group (comparison between different dose or FA alone and Vit-FA) (4) studies lack the necessary data (e.g., non-full text articles, unpublished trials).

### Data Extraction

Two investigators (YHY, XMS) assessed the eligibility of retrieved articles and extracted relevant data from each eligible study using a standardized form independently. From included studies, the following information were extracted: study characteristics (first author’s name, year of publication, study design, range of years in the studies, country), participants’ characteristics (population size, gestational age at recruitment, age, pre-conception BMI, FA dose, the initiation time of FA (pre-conception or post-conception), type of disease (GH or PE), the HRs, RRs, ORs with 95% CIs of GH/PE adjusted for confounders.

### Outcomes

The primary outcome was the risk of PE. The secondary outcome was the risk of GH.

### Quality Assessment

The methodological quality assessment of included studies was independently assessed by two reviewers (YHY, XMS) according to the Newcastle-Ottawa Scale (NOS) and the Cochrane Collaboration’s Risk of Bias Assessment Tool. Two reviewers (XRW, XF) resolved the disagreements, and the final consensus was reached by all four reviewers. For cohort studies, the NOS includes three aspects with a nine-point scale: selection of study population, compatibility of the study groups, and ascertainment of outcomes, and the scores of 0–3, 4–6, and 7–9 were interpreted as low, moderate, or high quality. For RCTs, the Cochrane Collaboration’s Tool includes six aspects with seven items: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias.

### Statistical analysis

This meta-analysis was conducted by Review Manager for Windows (version 5.3) using the generic inverse variance method. Between-study heterogeneity was quantified using Cochran’s  $Q$ -statistic and  $I^2$  statistics and a value of  $P < 0.10$  or an  $I^2 > 50\%$  was considered statistically significant for heterogeneity among studies. The random-effect model was used if it was significant; otherwise, the fixed-effect model was acceptable. Subgroup analyses were performed according to the type of disease (GH or PE), initiation time of FA (pre-conception or post-conception), form of FA (Vit-FA or FA alone) and pre-conception BMI ( $BMI < 25$  kg/m<sup>2</sup> or  $BMI \geq 25$  kg/m<sup>2</sup>), respectively. The sensitivity analysis was conducted by excluding the studies one by one to evaluate the stability of results without estimation bias from individual study. The funnel plots were used to evaluate publication bias qualitatively.

## RESULTS

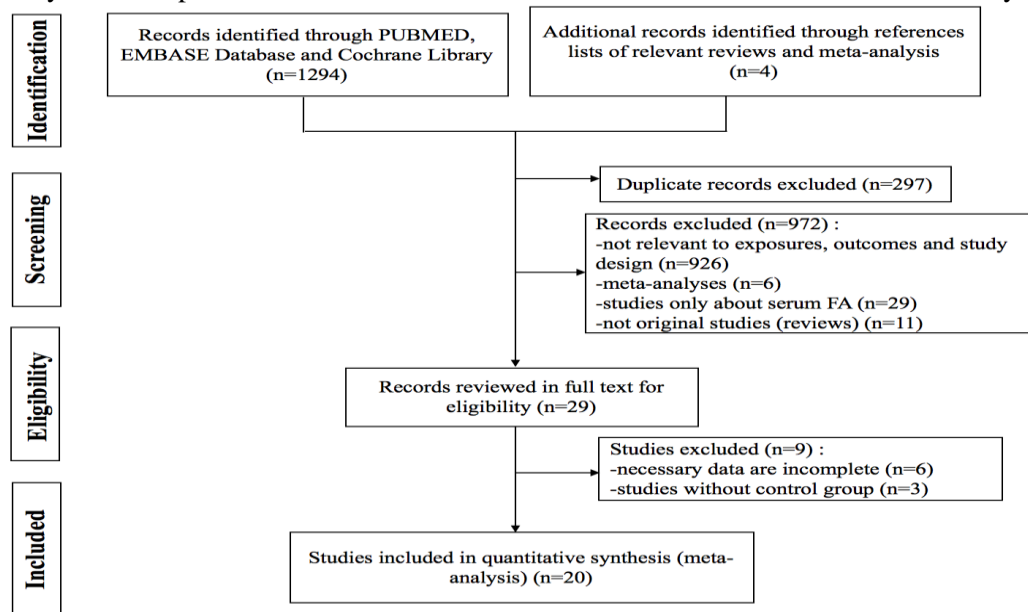
### Search Results

The search strategy yielded 1294 abstracts. Manual searching identified 4 additional articles. A total of 1269 articles were excluded after de-duplicating and the review of abstracts, leaving 29 studies for full-text review for eligibility. Overall, 20 studies with 359041 patients were identified that were eligible for inclusion in the meta-analysis.<sup>5-24</sup> The whole literature search process was summarized in the Figure 1.

## Study Characteristics

This meta-analysis included 3 RCTs and 17 cohort studies. Characteristics of the 20 included studies in the meta-analysis were presented in Table 1 and

Table 2. These studies were published between 2002 and 2020. The study populations ranged from 215 to 193554 participants, with a total number of 359041. There were 12 studies that didn't clearly describe the



**Figure 1.** Folic acid and the risk of Hypertensive Disorders of Pregnancy flow diagram.

initiation time of FA, 4 studies that began taking FA in post-conception period, and 1 study in pre-conception period. FA alone or Vit-FA was 0.2-1mg in most studies, except for 3 RCTs which used high dose (4-5mg) FA. The ascertainment of GH/PE were from medical records and self-report, and there are 14 studies evaluating the risk of PE alone and 6 studies evaluating the risk of both GH and PE. In addition, 5 articles performed a subgroup analysis based on the pre-conception BMI. The ORs, RRs and HRs in 18 studies were adjusted based on the potential confounders. The 20 studies were undertaken in different countries, including the USA (n=6), Canada (n=4), China (n=4), Denmark (n=2), Netherlands (n=1), the UK (n=1), South Korea (n=1) and Australia (n=1).

## Quality Assessment

NOS was used to assess the risk of bias for 17 cohort studies. All cohort studies were given a score of 7-9 stars, representing the high quality of studies. The six cohort studies had scores of 7-8 for the reason that FA and HDP were identified only based on self-reports. The results of this quality assessment were summarized in Appendix 2-Table A. Cochrane Collaboration's Tool was used to assess the risk of bias for 3 RCTs, and only random sequence generation of Charles 2005 was unclear.<sup>6</sup> Appendix 3-Figure 1 and Figure 2 showed the results of the risk of bias assessment, representing that all RCTs were of high quality.

## High Dose (HD)-FA and the Risk of PE

Three studies compared risk of PE with HD-FA. The results showed that HD-FA was not associated with PE (3 studies, RR 1.11, 95% CI 0.75-1.62,  $P=0.61$ ; Figure 2). No obvious asymmetry was found in the funnel plot (Appendix 4-Figure 1), which indicated that publication bias was unlikely in the analysis. The results of sensitivity analysis showed no substantial modification of the estimates after exclusion of individual study one by one.

## Low Dose (LD)-FA and the Risk of HDP(GH/PE)

Eighteen studies compared risk of HDP with LD-FA. Stratification by GH and PE showed that LD-FA significantly decreased the risk of PE (18 studies, RR 0.83, 95% CI 0.74-0.93,  $P=0.0008$ , Figure 3), but was not associated with GH (6 studies, RR 1.05, 95% CI 0.97-1.13,  $P=0.20$ ; Figure 3). No obvious asymmetry was found in the funnel plot (Appendix 4-Figure 2), which indicated that publication bias was unlikely in the analysis. The results of sensitivity analysis showed that the association between LD-FA and decreased risk of PE in PE subgroup was reliable.

## Pre-conception or Post-conception LD-FA and the Risk of PE

11 studies with 23 results compared the association between peri-conception LD-FA and the risk of PE. According to the initiation time (Pre-conception or Post-conception), a subgroup analysis was

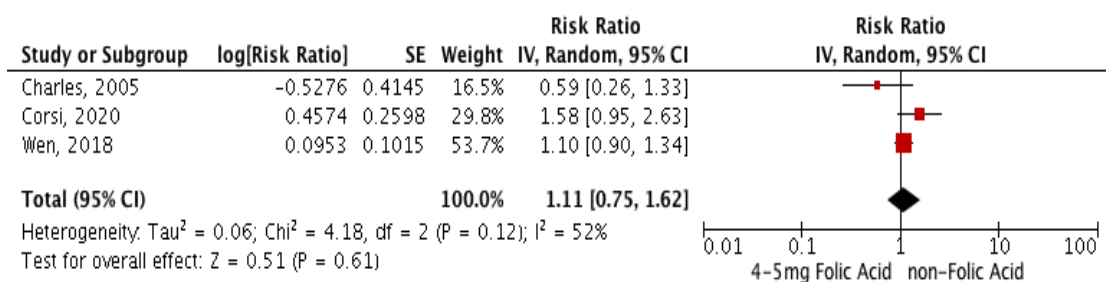


Figure 2. Forest plot for High Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy

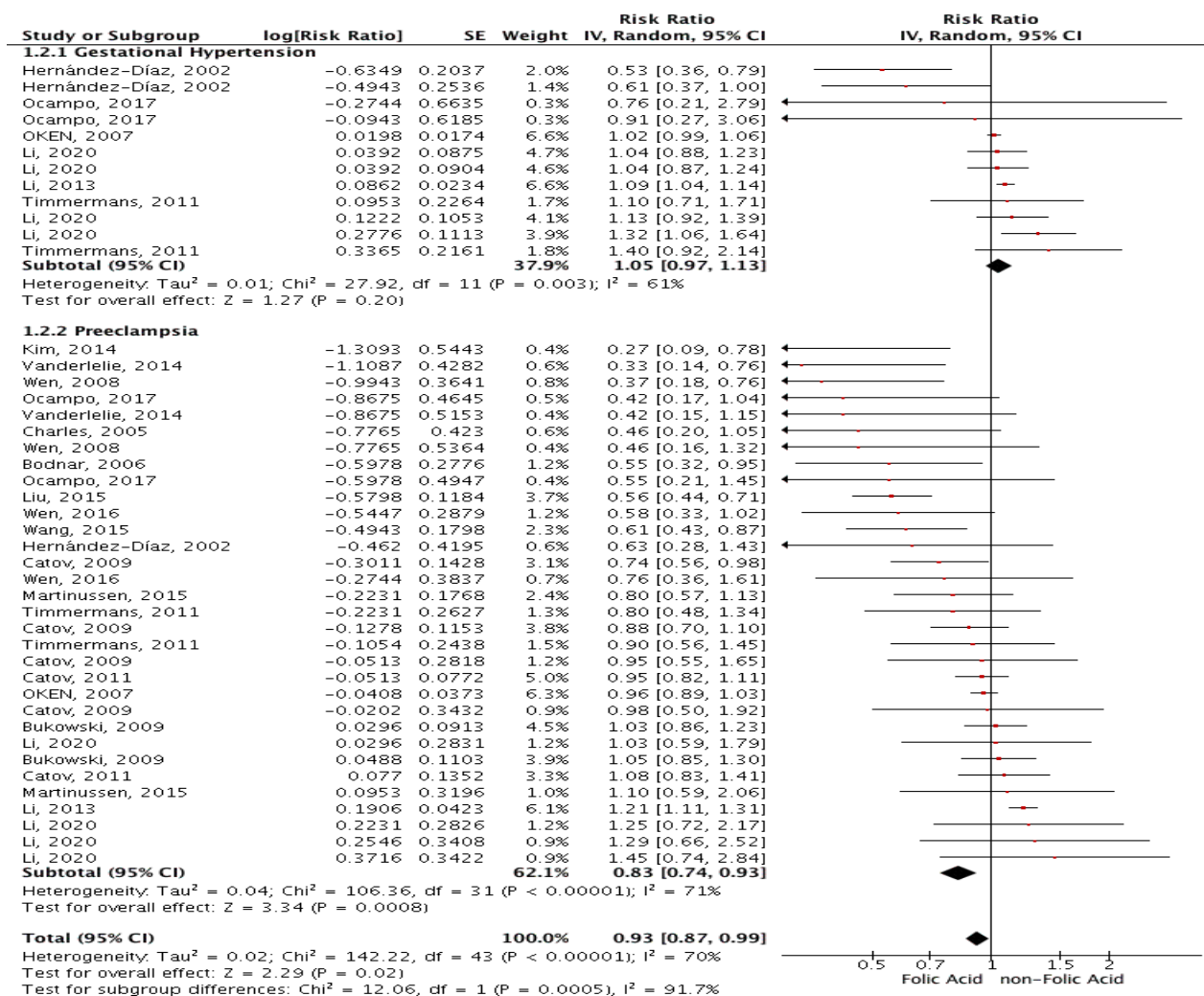


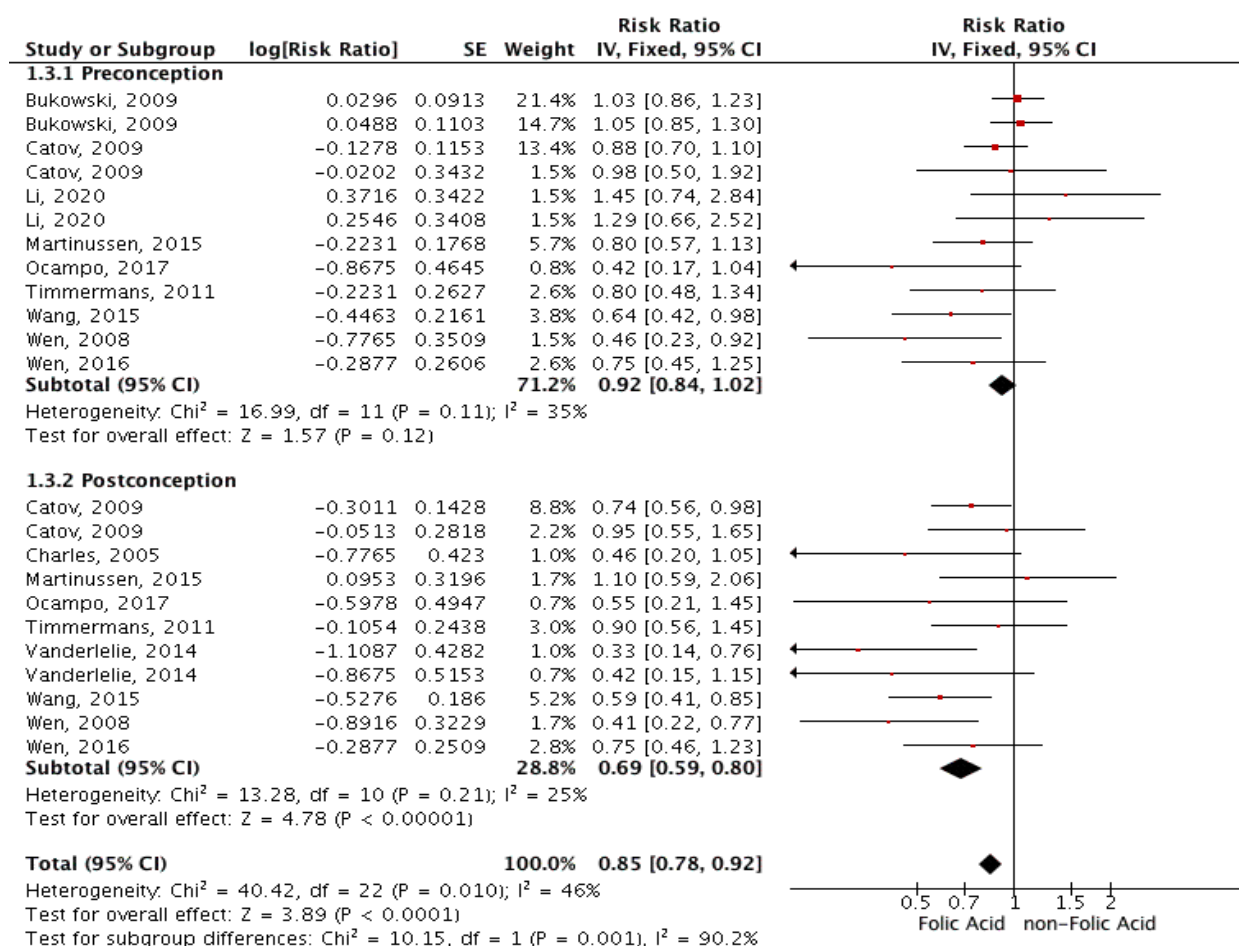
Figure 3. Forest plot for Low Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy in subgroup analysis by type of disease (Gestational hypertension or Pre-eclampsia)

performed. The results showed that pre-conception LD-FA was not associated with the PE (9 studies, RR 0.92, 95% CI 0.84-1.02, P=0.12, Figure 4), but post-conception LD-FA had a 31% decreased risk of PE (9 studies, RR 0.69, 95% CI 0.59-0.80, P<0.00001; Figure 4). No obvious asymmetry was found in the funnel plot (Appendix 4-Figure 3), which indicated that publication bias was unlikely in the analysis. The results of sensitivity analysis showed that the

association between post-conception LD-FA and the decreased risk of PE was reliable.

**LD-FA in patients with pre-conception BMI<25 kg/m<sup>2</sup> or BMI≥25 kg/m<sup>2</sup> and the Risk of PE**

According to the pre-conception BMI (BMI<25 kg/m<sup>2</sup> or BMI≥25 kg/m<sup>2</sup>), a subgroup analysis was performed to compare the association between LD-



**Figure 4.** Forest plot for Low Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy in subgroup analysis by initiation time (pre-conception or post-conception)

FA and the risk of PE in 4 studies with 14 results. The results showed that LD-FA in patients with pre-conception  $\text{BMI} < 25 \text{ kg/m}^2$  had a 32% decreased risk of PE (4 studies, RR 0.68, 95%CI 0.56-0.81,  $P < 0.0001$ , Figure 5), but no difference was found in patients with pre-conception  $\text{BMI} \geq 25 \text{ kg/m}^2$  (4 studies, RR 0.87, 95%CI 0.71-1.05,  $P = 0.14$ ; Figure 5). No obvious asymmetry was found in the funnel plot (Appendix 4-Figure 4), which indicated that publication bias was unlikely in the analysis. The results of sensitivity analysis showed that the association between LD-FA and the decreased risk of PE in pre-conception  $\text{BMI} < 25 \text{ kg/m}^2$  subgroup was reliable.

#### FA alone or Vit-FA and the Risk of PE

According to the form of FA (FA alone or Vit-FA), a subgroup analysis was performed in 8 studies with 13 results. The results showed that Vit-FA significantly decreased the risk of PE (4 studies, RR 0.82, 95%CI 0.68-0.98,  $P = 0.03$ , Figure 6), but no reduction in PE was found in patients receiving FA

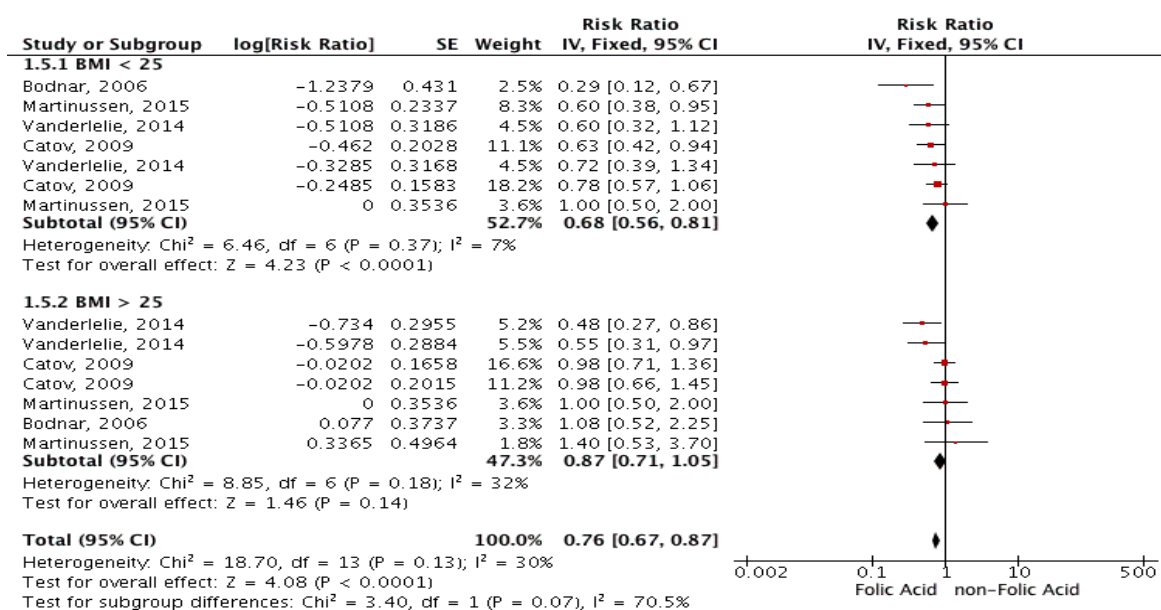
alone (7 studies, RR 0.86, 95%CI 0.65-1.13,  $P = 0.27$ ; Figure 6). Asymmetry can be seen in the funnel plot

(Appendix 4-Figure 5), especially the Vit-FA subgroup, indicating that there is publication bias in the analysis. In addition, after excluding *Bodnar 2006*,  $P$ -value changed to 0.86 (RR, 95%CI 0.73-1.01,  $P = 0.06$ ), after excluding *Catov 2009*,  $P$ -value changed to 0.83 (RR, 95%CI 0.66-1.04,  $P = 0.10$ ), which indicated that the association between Vit-FA and the decreased risk of PE was not reliable.

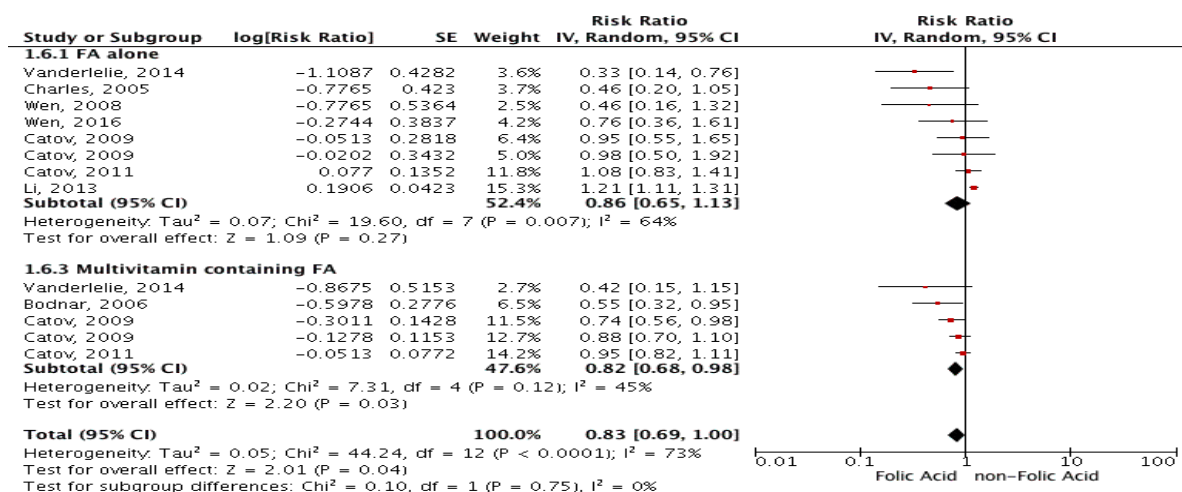
#### DISCUSSION

Our meta-analysis of 20 studies involving 359041 patients found that LD-FA was associated with the decreased risk of PE but not GH. In addition, post-conception LD-FA and pre-conception  $\text{BMI} < 25 \text{ kg/m}^2$  were considered as protective factors to reduce the risk of PE.

Some studies of the association between FA and the risk of GH or PE have shown a potential protective effect (5, 7, 9, 11, 15-17, 19), and Wen



**Figure 5.** Forest plot for Low Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy in subgroup analysis by pre-conception Body Mass Index (BMI<25 kg/m<sup>2</sup> or BMI≥25 kg/m<sup>2</sup>)



**Figure 6.** Forest plot for Low Dose Folic Acid and the risk of Hypertensive Disorders of Pregnancy in subgroup analysis by form of Folic Acid (Multivitamin containing FA or FA alone)

(22) has shown that maternal exposure to FA antagonists appears to increase the risk of placenta-mediated adverse outcomes of pregnancy (33). It has been proved that elevated plasma homocysteine (Hcy) level was associated with increased risk for various forms of CVD, and it was also significantly elevated in women with PE (34). The possible pathogenic mechanisms involve damage of endothelial cell, reduction of vascular flexibility, changes in the hemostatic process and promotion of the development of inflammation. The elevated Hcy may arise from genetic defect of blood pressure, the altered process of DNA and protein synthesis of placental growth and development, and the imbalance between FA and vitamin B<sub>12</sub> (24).

methylenetetrahydrofolate reductase (MTHFR) or insufficient intake of FA (35-36). In the prevention and treatment of CVD, in addition to reducing Hcy levels, FA could also increase nitric oxide synthase (NOS) coupling and nitric oxide (NO) bioavailability to improve vascular health and function which are independent of the Hcy-lowering effect (37). However, two studies have shown that FA supplementation might be associated with the increased risk of GH or PE (14, 24). There are some possible explanations, including the increased red cell blood mass which is correlated with elevated

In the late first trimester or early second trimester, placental growth and development reach the peak. Thus, FA supplementation in this period

**Table 1.** The characteristics of included studies

Author, year	Design, years, country	Characteristics	FA initiation time	Dose	Gestational age (weeks) at recruitment	Age(years)	Pre-conception BMI(kg/m <sup>2</sup> )
Li, 2020	Prospective cohort, 2013-, China	4853 participants from the Tongji Maternal and Child Health Cohort with information on periconceptional FA supplement use and diagnosis of GH/PE, 1161 and 161 women were diagnosed with GH and PE	pre-conception or post-conception	at least 0.4mg/day	NR	28.6 (SD 3.4)	20.6 (SD 2.6)
Corsi, 2020	Randomized Controlled Trial, 2011-2015, Canada	428 women pregnant with twins who were aged 18 y or older, between 8 and 16 completed weeks' gestation and had at least one high risk factor for PE	post-conception	4mg/day	13.8 (SD 1.9,FA) 14.0 (SD 1.8,non-FA)	31.5 (SD5.2, FA) 31.4 (SD5.0,non-FA)	NR
Wen, 2018	Randomized Controlled Trial, 2011-2015, Canada	2301 pregnant women with at least one high risk factor for PE (1144 to the FA group and 1157 to the placebo group)	post-conception	4mg/day	14 (SD 1.9)	31 (SD 5.4)	34 (SD 8.6, FA) 34 (SD 13, non-FA)
Ocampo, 2017	Retrospective cohort, 2004-2014, USA	3247 participants recruited from the Mother-ToBaby (MTB) network, 2697 were early users and 471 were late users of FA-containing supplements, and 79 were nonusers during their pregnancy	pre-conception or post-conception	NR	NR	32.3 (SD 4.7, pre-conception-FA) 30.4 (SD 6.0, post-conception-FA) 32.8 (SD 5.6,non-FA)	24.5 (SD 5.2, pre-conception FA) 25.9 (SD 6.1, post-conception FA) 25.7 (SD 7.3,non-FA)
Wen, 2016	Prospective cohort, 2002-2008, Canada	7669 pregnant women who were less than 20 weeks gestational age in Ottawa, ON and Kingston, ON, Canada	pre-conception or post-conception	most 1.0 mg or higher	NR	NR	NR
Liu, 2015	Prospective cohort, 2010-2012, China	10179 women (7864 FA supplement, 2315 non-FA) with live singleton births in Lanzhou, China	pre-conception or post-conception	NR	NR	NR	NR
Wang, 2015	Prospective cohort, 2010-2012, China	10041 pregnant women with gestational age $\geq$ 20 weeks and aged 18 years or older, without chronic hypertension, gestational hypertension and mental illness	pre-conception or post-conception	NR	NR	NR	NR
Martinussen, 2015	Prospective cohort,1996-2000, USA	3647 women (1710 from the AIP study and 1937 from the NIP study) who were followed from the first trimester of pregnancy in New England, USA	pre-conception or post-conception	NR	NR	NR	NR

*Table 1 continues...*

Kim, 2014	Retrospective cohort, 2009-2010, South Korea	215 participants with singleton pregnancies at the Korea University Anam Hospital	NR	NR	NR	31.9 (SD 3.9,FA) 31.3 (SD 4.7,non-FA)	NR
Vanderlelie, 2014	Prospective cohort, 2006-2011, Australia	2619 mothers with live births in Queensland, Australia	post-conception	most 0.8 mg/day	NR	NR	NR
Li, 2013	Retrospective cohort, 1993-1995, China	193554 women in 2 southern provinces (Jiangsu Province and Zhejiang Province)	pre-conception or post-conception	0.4mg/day	NR	24.21 (SD 2.47,FA) 25.54 (SD 3.77,non-FA)	20.37 (SD 2.29,FA) 20.89 (SD 2.49,non-FA)
Timmermans, 2011	Prospective cohort, 2002-2006, Netherlands	9778 women at early pregnancy in Rotterdam, the Netherlands	pre-conception or post-conception	0.4-0.5mg/day	NR	29.8 (IQR 19.7-39.0, post-conception) 31.6 (IQR 22.6-39.5, pre-conception) 27.8 (IQR 17.9-39.2, non-FA)	NR
Catov, 2011	Prospective cohort, 1997-2003, Denmark	35897 pregnant women in the Danish National Birth Cohort	pre-conception or post-conception	Vit-FA: most FA0.2mg/day	10.7 (SD 3.5,FA) 10.9 (SD 3.8, Vit-FA) 11.4 (SD 4.3,non-FA)	NR	NR
Catov, 2009	Prospective cohort, 1997-2003, Denmark	28601 pregnant women in the Danish National Birth Cohort	pre-conception or post-conception	FA: 0.4mg/day Vit-FA: most FA0.2mg/day	10.8 (SD 3.4,FA) 10.9 (SD 3.5 Vit-FA) 11.2 (SD 3.8,non-FA)	NR	NR
Bukowski, 2009	Prospective cohort, 1999-2002, USA	34480 women who delivered singleton pregnancies with a gestational age of 10 week 3 d through 13 week 6 d	pre-conception	NR	NR	27.8 (IQR 23.5–32.5,non-FA) 31.1 (IQR 27.1–34.5,<1 year) 33.1 (IQR29.4–36.3,>1 year)	NR
Wen, 2008	Prospective cohort, 2002-2005, Canada	2951 pregnant women with prenatal care visit (12-20 weeks' gestation) at the Ottawa Hospital and Kingston General Hospital	pre-conception or post-conception	most 1.0 mg or higher	NR	NR	NR
Oken, 2007	Prospective cohort, 1999-2002, USA	1718 participants with live births in Project Viva	NR	normal women: 0.952(SD 0.467)mg PE: 0.854(SD 0.297)mg GH: 1.019(SD 0.486)mg	10.4	NR	NR

Table 1 continues...



Bodnar, 2006	Prospective cohort, 1997-2001, USA	1835 women aged 14–44 years, carrying singleton infants, and planning to deliver at Magee-Womens Hospital	NR	NR	NR	NR	NR
Charles, 2005	Randomized Controlled Trial, 1996-1967, UK	2928 pregnant women with live and stillborn singletons in Aberdeen City	post-conception	LD:0.2mg/day HD:5mg/day	17.5 (SD 5.3,non-FA) 16.9 (SD 4.8,LD-FA) 17.7 (SD 5.6,HD-FA)	26.0 (SD 5.6,non-FA) 25.8 (SD 5.5,LD-FA) 25.5 (SD 5.2,HD-FA)	NR
Hernández-Díaz, 2002	Retrospective cohort, 1993-2000, USA	2100 women with nonmalformed infants who did not have chronic hypertension of the Slone Epidemiology Center	pre-conception or post-conception	at least 0.4 mg	NR	NR	23.9 (SD 4.7)

**FA**, folic acid; **Vit-FA**, multivitamin containing folic acid; **HD-FA**, high dose folic acid; **LD-FA**, low dose folic acid; **GH**, gestational hypertension; **PE**, pre-eclampsia; **BMI**, Body Mass Index; **IQR**, interquartile range; **SD**, standard deviation; **NR**, not reported.

**Table 2.** The clinical outcomes of included studies

Author, year	Outcome-GH/PE	FA (n)	FA-GH/PE (n)	non-FA (n)	non-FA-GH/PE (n)	FA-GH/PE risk RR/OR/HR-95% CI	Adjusted for
Li, 2020	GH-FA800-pre-conception	358	107	646	145	1.32 (1.06-1.64)	age, education, work status, monthly income, ethnicity, history of abnormal pregnancy, pre-conception BMI, smoking, drinking, family history of diabetes mellitus, family history of hypertension, other supplement use, gestational diabetes mellitus, and gestational weight gain
	PE-FA800-pre-conception	358	17	646	18	1.45 (0.74-2.83)	
	GH-FA400-pre-conception	573	143	646	145	1.13 (0.92-1.39)	
	PE-FA400-pre-conception	573	19	646	18	1.29 (0.66-2.51)	
	GH-FA800-post-conception	1504	358	646	145	1.04 (0.87-1.24)	
	PE-FA800-post-conception	1504	50	646	18	1.03 (0.59-1.79)	
	GH-FA400-post-conception	1772	408	646	145	1.04 (0.88-1.24)	
Corsi, 2020	PE	215	37	213	21	1.25 (0.72-2.18)	age, smoking, parity, country, use of aspirin, and history of preeclampsia
	PE	1174	169	1157	156	1.58 (0.95-2.63)	
Wen, 2018	PE	1174	169	1157	156	1.10 (0.90-1.34)	parity, maternal age, and cigarette smoking
Ocampo, 2017	GH-pre-conception	2697	95	79	3	0.91 (0.27-3.05)	education, race/ethnicity, parity, pre-conception BMI, diabetes status, asthma status, autoimmune disease status, cigarettes smoked per day, maternal age, gravidity, previous spontaneous abortion or stillbirth, antidepressant use, cohort study and enrollment year
	PE-pre-conception	2697	94	79	6	0.42 (0.17-1.05)	
	GH-post-conception	471	14	79	3	0.76 (0.21-2.83)	
	PE-post-conception	471	29	79	6	0.55 (0.21-1.46)	

Table 2 continues...

Wen, 2016	PE-FA	625	24	404	17	0.76 (0.36-1.62)	maternal age, previous health problem (chronic hypertension, history of preeclampsia, diabetes), smoking, and parity
	PE- Vit-FA +FA	7265	228	404	17	0.58 (0.33-1.02)	
	PE-pre-conception	3034	96	404	17	0.75 (0.45-1.25)	
	PE-post-conception	4018	127	404	17	0.75 (0.46-1.23)	
Liu, 2015	PE	7864	206	2315	109	0.56 (0.44-0.70)	NR
Wang, 2015	PE	794	238	265	115	0.61 (0.43-0.87)	maternal age, education level, parity, maternal diabetes, pre-conception BMI, weight gain during pregnancy, family monthly income per capita, multiple birth, maternal employment during pregnancy, history of gestational hypertension and dietary folate intake
	PE-pre-conception	306	97	265	115	0.64 (0.42-0.98)	
	PE-post-conception	450	130	265	115	0.59 (0.41-0.85)	
Martinussen, 2015	PE-pre-conception	1590	NR	2057	NR	0.80 (0.60-1.20)	study (AIP or NIP), maternal age, maternal ethnicity, maternal education, maternal marital status, parity, maternal smoking in pregnancy and any miscarriages or stillbirths in previous pregnancies
	PE-post-conception	3301	NR	346	NR	1.10 (0.60-2.10)	
	BMI<25-PE-pre-conception					0.60 (0.40-1.00)	
	BMI<25-PE-post-conception	NR	NR	NR	NR	1.00 (0.50-2.00)	
	BMI>25-PE-pre-conception	NR	NR	NR	NR	1.00 (0.50-2.00)	
	BMI>25-PE-post-conception	NR	NR	NR	NR	1.40 (0.50-3.50)	
Kim, 2014	PE	134	6	81	12	0.27 (0.09-0.76)	parity, familial monthly income, preeclampsia and prenatal intake of folic acid
Vanderlelie, 2014	PE-FA	476	6	1066	31	0.33 (0.14-0.75)	maternal age, parity, gestational diabetes, indigenous status and maternal smoking
	PE- Vit-FA	719	7	1066	31	0.42 (0.13-0.98)	
	BMI<25-PE-FA	283	4	616	12	0.72 (0.39-1.35)	
	BMI<25-PE- Vit-FA	406	4	616	12	0.60 (0.39-1.36)	
	BMI>25-PE-FA	193	5	450	21	0.55 (0.31-0.96)	
	BMI>25-PE- Vit-FA	313	3	450	21	0.48 (0.27-0.86)	
Li, 2013	GH	32919	3226	100823	9477	1.09 (1.04-1.14)	age (continuous), body mass index (continuous), education, occupation, parity, and multiple births
	PE	32919	888	100823	2420	1.21 (1.11-1.31)	
Timmermans, 2011	GH-pre-conception	2362	165	1770	48	1.10 (0.70-1.70)	gestational age, age, BMI, parity, ethnicity, education, smoking, alcohol consumption, antenatal care
	GH-post-conception	1861	87	1770	48	1.40 (0.90-2.10)	
	PE-pre-conception	2362	45	1770	39	0.80 (0.50-1.40)	
	PE-post-conception	1861	41	1770	39	0.90 (0.50-1.30)	
Catov, 2011	PE-FA	2609	65	11503	265	1.08 (0.83-1.41)	NR
	PE-Vit-FA	21789	479	11503	265	0.95 (0.82-1.11)	
Catov, 2009	PE-pre-conception- Vit-FA	6655	NR	7582	NR	0.88 (0.70-1.10)	body mass index, smoking, parity, chronic hypertension, gestational age at recruitment, and partial multivitamin use
	PE-post-conception- Vit-FA	3666	NR	7582	NR	0.74 (0.56-0.98)	
	PE-pre-conception-FA	NR	NR	NR	NR	0.98 (0.50-1.92)	
	PE-post-conception-FA	NR	NR	NR	NR	0.95 (0.55-1.66)	
	BMI<25-PE-pre-conception-Vit-FA	NR	NR	NR	NR	0.78 (0.57-1.06)	
	BMI<25-PE-post-conception-Vit-FA	NR	NR	NR	NR	0.63 (0.42-0.93)	

Table 2 continues...

	BMI>25-PE-pre-conception-Vit-FA	NR	NR	NR	NR	0.98 (0.71-1.36)	
	BMI>25-PE-post-conception-Vit-FA	NR	NR	NR	NR	0.98 (0.59-1.30)	
Bukowski, 2009	PE-pre-conception<1year	12444	295	15259	381	1.03 (0.86-1.23)	maternal age, body mass index, race and ethnicity, educational level, marital status, smoking, parity and history of prior preterm birth
	PE-pre-conception>1year	6777	157	15259	381	1.05 (0.85-1.31)	
Wen, 2008	PE-Vit-FA+FA	2713	59	238	12	0.37 (0.18-0.75)	maternal age, ethnic background, educational level, parity, previous preeclampsia, chronic hypertension, diabetes, pre-conception body mass index, household income, gestational age at recruitment, and cigarette smoking during pregnancy
	PE-FA	421	12	238	12	0.46 (0.16-1.31)	
	PE-pre-conception	1031	24	238	12	0.46 (0.23-0.91)	
	PE-post-conception	1682	35	238	12	0.41 (0.22-0.78)	
Oken, 2007	GH	NR	NR	NR	NR	1.02 (0.99-1.06)	maternal age (<20, 20-40, 40+ years), pre-conception body mass index (continuous), first-trimester systolic blood pressure (continuous), race/ethnicity (black, Hispanic, white, other), education (college graduate, < College graduate), and parity (0, 1+)
	PE	NR	NR	NR	NR	0.96 (0.89-1.03)	
Bodnar, 2006	PE-Vit-FA	860	33	975	43	0.55 (0.32-0.95)	race (Black, other), marital status (married, unmarried), parity (primiparous, multiparous), pre-conceptional physical activity (yes, no), and poverty index ratio (130%, 131-299%, 300%)
	BMI<25-PE- Vit-FA	860	21	975	42	0.29 (0.12-0.65)	
	BMI>25-PE- Vit-FA	860	53	975	45	1.08 (0.52-2.25)	
Charles, 2005	PE-LD	448	8	1890	51	0.46 (0.20-1.05)	smoking at booking, social class, parity, gestation at booking, height of women, weight of women at booking and gestation at delivery
	PE-HD	459	7	1890	51	0.59 (0.26-1.32)	
Hernández-Díaz, 2002	GH	NR	NR	NR	NR	0.53 (0.36-0.80)	geographic region, family income, and maternal age, pre-conception weight, parity, twin pregnancy, diabetes, smoking, and education
	PE	NR	NR	NR	NR	0.63 (0.28-1.45)	
	GH-pre-conception	NR	NR	NR	NR	0.61 (0.37-1.00)	

**FA**, folic acid; **Vit-FA**, multivitamin containing folic acid; **HD-FA**, high dose folic acid; **LD-FA**, low dose folic acid; **GH**, gestational hypertension; **PE**, pre-eclampsia; **BMI**, Body Mass Index; **HR**, hazard ratio; **OR**, odds ratio; **RR**, relative risk; **CI**, confidence interval; **NR**, not reported

may be most effective to prevent PE which was consistent with our finding that post-conception FA could reduce the risk of PE rather than pre-conception FA (22). It should be noted that pre-conception FA is a necessary measure to prevent neural tube defects (NTDs). Therefore, it is a more clinically appropriate choice to extend the use of FA into the later trimesters rather than starting it after pregnancy.

Another finding from our analysis was that FA supplementation could have a significant protective effect among women with pre-conception BMI < 25 kg/m<sup>2</sup> but not statistically significant among overweight women (pre-conception BMI ≥ 25 kg/m<sup>2</sup>). Firstly, the pathogenic mechanisms of PE may be different among women with BMI < 25 kg/m<sup>2</sup> and BMI ≥ 25 kg/m<sup>2</sup>. The excess risk of PE might be associated with overweight or even obese status. Additionally, most pregnant women take low dose of FA, but both FA alone and multivitamin may not be adequate to overcome the metabolic disturbances of overweight or obese status (7, 11).

Our meta-analysis had several advantages. First, RCTs of HD-FA supplementation were analyzed separately from LD-FA to avoid bias, as the relationship between HD-FA and PE is still controversial. In addition, we did the subgroup analysis and the results demonstrated that post-conception LD-FA and pre-conception BMI < 25 kg/m<sup>2</sup> were protective factors for pregnant women. We also acknowledged some limitations in this study. First, it has been recommended for women planning pregnancy to use FA until the third month of pregnancy to prevent NTDs. However, the discontinued time of FA is unclear for the pregnant women included in this study, particularly pre-conception FA. Therefore, the association of post-conception FA and the decreased risk of PE need to be further confirmed. Secondly, most studies included in this study did not consider the effects of serum FA and serum Hcy. Meanwhile, the genotype of MTHFR was not considered in most studies but it was also an important factor because the studies included came from different countries. And it was difficult to accurately evaluate the dose of dietary FA, which could cause the bias of final results. In addition, although the result indicated that Vit-FA might be more meaningful than FA alone to reduce the risk of PE, it was hard to further compare the effects of FA with Vit-FA due to the few clinical studies. Lastly, only few studies have been conducted in pregnant women with high-risk (20, 22-23). Thus, we need more high-quality clinical studies to confirm the findings of this study, such as detailed documentation for FA dose, start and end

time, form of FA, high-risk factors, and detection of genotype of MTHFR and serum FA.

## CONCLUSIONS

Our meta-analysis found that LD-FA tended to decrease the risk of PE. In addition, post-conception LD-FA and pre-conception BMI < 25 kg/m<sup>2</sup> were considered as protective factors to reduce the risk of PE. Therefore, we recommend that pregnant women use LD-FA throughout the pregnancy period to avoid the occurrence of PE. More importantly, a more convincing result requires the inclusion of more rigorous RCTs.

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## CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## SUPLIMENTARY DATA

### Appendix 1

#### PUBMED:251

("vitamin b9"[Text Word] OR "folic acid"[Text Word]) AND (("preeclampsia"[Text Word] OR "pregnancy induced hypertension"[Text Word]) OR "gestational hypertension"[Text Word])

#### EMBASE: 950

1	*folic acid/ or vitamin b9.mp.	23490
2	folic acid.mp.	77966
3	*preeclampsia/ or preeclampsia.mp.	63544
4	gestational hypertension.mp. or *maternal hypertension/	9880
5	pregnancy induced hypertension.mp.	5371
6	hypertensive disorders of pregnancy.mp.	3420
7	1 or 2	78075
8	3 or 4 or 5 or 6	70227
9	7 and 8	950

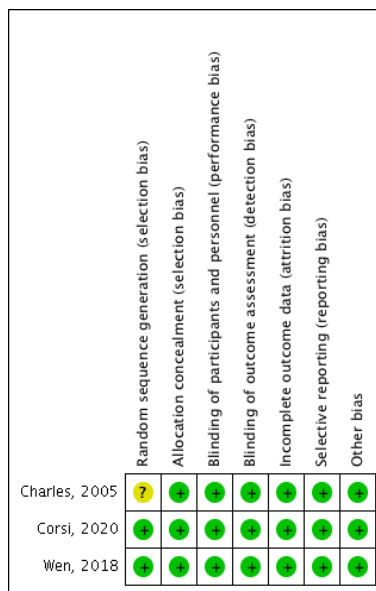
#### COCHRANE: 93

- #1 (vitamin b9):ti, ab, kw
- #2 MeSH descriptor: [Folic Acid] this term only
- #3 (folic acid):ti, ab, kw
- #4 (#1) OR (#2) OR (#3)
- #5 MeSH descriptor: [Pre-Eclampsia] this term only
- #6 (preeclampsia):ti, ab, kw
- #7 (pre-eclampsia):ti, ab, kw
- #8 MeSH descriptor: [Hypertension, Pregnancy-Induced] this term only
- #9 (pregnancy induced hypertension):ti, ab, kw
- #10 (gestational hypertension):ti, ab, kw
- #11 (#5) OR (#6) OR (#7) OR (#8) OR (#9 OR #10)
- #12 (#11) AND (#4)

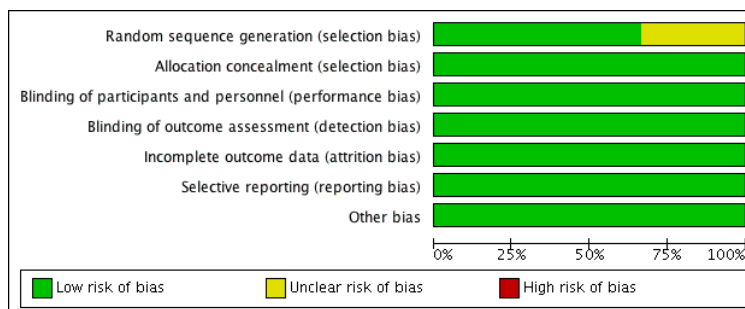
**Appendix 2. Table A. Quality of observational studies (indicators from New-Castle-Ottawa scale)**

study	1 <sup>a</sup>	2 <sup>b</sup>	3 <sup>c</sup>	4 <sup>d</sup>	5A <sup>e</sup>	5B <sup>f</sup>	6 <sup>g</sup>	7 <sup>h</sup>	8 <sup>i</sup>	Total quality scores
Li, 2020	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Ocampo, 2017	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Wen, 2016	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	8
Liu, 2015	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	7
Wang, 2015	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Martinussen, 2015	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Kim, 2014	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	8
Vanderlelie, 2014	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Li, 2013	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Timmermans, 2011	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Catov, 2011	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	7
Catov, 2009	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Bukowski, 2009	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	7
Wen, 2008	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Oken, 2007	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Bodnar, 2006	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Hernández-Díaz, 2002	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	8

- a: Indicates exposed cohort truly representative
- b: Non-exposed cohort drawn from the same community
- c: Ascertainment of exposure from the same community
- d: Outcome of interest not present at start of study
- e: Cohorts comparable on basis of site and etiology of infection
- f: Cohorts comparable on others factors
- g: Assessment of outcome of record linkage or independent blind assessment
- h: Follow-up long enough for outcomes to occur
- I: Complete accounting for cohort

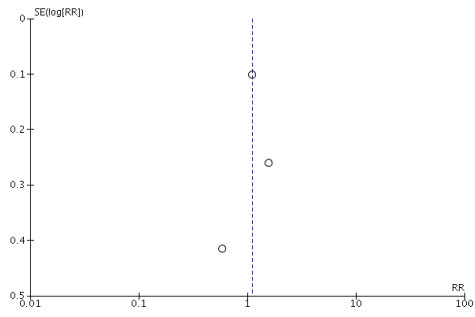


Appendix 3 Figure 1

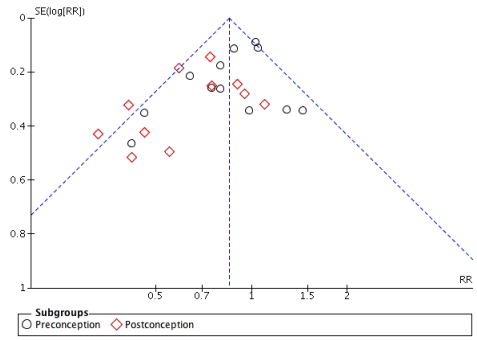


Appendix 3 Figure 2

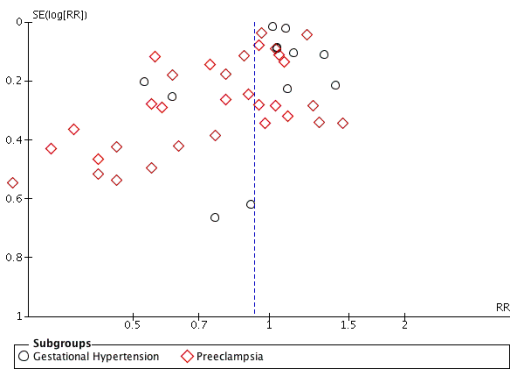




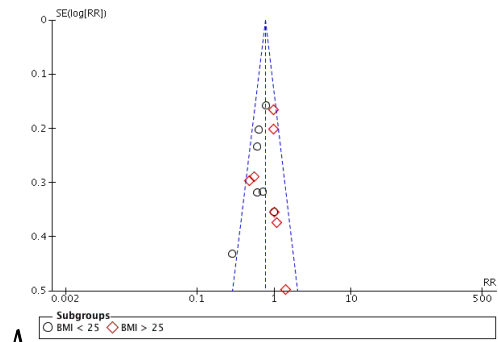
Appendix 4 Figure 1



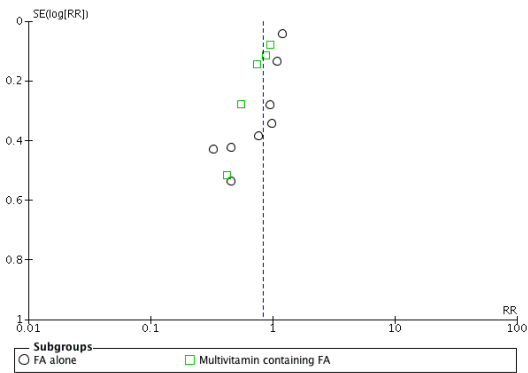
Appendix 4 Figure 4



Appendix 4 Figure 2



Appendix 4 Figure 5



Appendix 4 Figure 3

