

## Supplementary material

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### APPENDIX 1. PROTOCOL

	<b>UNIVERSIDAD NACIONAL DE COLOMBIA</b>	
	<b>RESEARCH PROJECT FORMULATION AND EXECUTION</b>	
<b>Protocol information</b>		
<b>Title:</b> Effectiveness and safety of congenital syphilis treatment in the last trimester in pregnant women with a confirmed diagnosis of syphilis: Systematic review. Protocol		
<b>Researchers</b>		
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* Name of the Research Group (GrupLAC) Health Technology and policies Assessment Group	Date: 30/11/23	Number of researchers: 3
*Research subarea: Sexually transmitted infections		
<b>School:</b> School of Medicine, Universidad Nacional de Colombia		
<b>Assessor</b>		
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<b>Department:</b>		
<b>Keywords:</b> congenital syphilis, drug treatment, penicillin G, effectiveness, controlled clinical trial, adverse effects		
<b>Submission date:</b> 30/11/23		

### 1. DESCRIPTION OF THE PRELIMINARY PROJECT:

Formulation of the research problem:

Gestational syphilis is defined as infection by *Treponema pallidum* acquired during pregnancy or a previously infected woman who becomes

pregnant. This condition can affect the foetus due to transplacental transmission of *Treponema*, which corresponds to what we know as congenital syphilis, this infection increases the risk of abortion, foetal death, preterm birth, low birth weight, neonatal infection, early neonatal death

and congenital malformations (1). GS and CS are on the rise in Colombia; the National Institute of Health has reported an increase between 2016 and 2020 of gestational and congenital syphilis, with a prevalence ratio that increased from 6.6 x 1000 live births (LB) to 13.5 x 1000 LB and congenital syphilis from 1.11 to 2.17 x 1000 LB respectively (2). Diagnosis and treatment of GS are the same as in non-pregnant women and me., based on screening with non-treponemal test (NTT) and confirmation with treponemal tests (TT) in the usual diagnostic strategy or with the inverse strategy that is used more in high-risk population or at scenarios of low setting resources. Benzathine penicillin is the election treatment for T pallidum infection and the dose depends on the stage of syphilis (3).

The opportune detection and treatment GS is a relatively simple and cost-effective intervention, even in low-resource settings, in preventing adverse outcomes associated with CS (4). The strategy of early diagnosis with rapid tests and immediate treatment (on the same day) with at least one dose of benzathine penicillin leads to a reduction of about 98% in the incidence of CS (relative risk (RR), 0.02; 95% confidence interval (CI: 0.00-0.18) and a reduction of half the risk of perinatal death (RR, 0.50; 95% CI: 0.33-0.84) (5) as long as treatment for GS been administered 30 days before delivery (5). Other treatment options that have been evaluated, such as doxycycline and tetracyclines, are contraindicated in pregnancy because they are potentially toxic to the foetus (category D) (6). Regarding azithromycin, an antibiotic of the macrolide class, classified as category B in pregnancy, azithromycin has been detected in the umbilical cord and amniotic fluid (6); however, some studies indicate that macrolides do not adequately cross the placental barrier (7, 8). Regarding Ceftriaxone, there are no adequate studies to assess its effects in women with gestational syphilis (9). To date, no alternative therapies to penicillin have been described that reliably cross the placenta to treat both the mother and the foetus (10)

Penicillins are bactericidal drugs that inhibit the synthesis of the bacterial peptidoglycan cell wall during active multiplication, killing susceptible bacteria (11). To date, penicillin remains effective for managing syphilis, with no evidence of resistance. Crystalline penicillin has been administered over time as a treatment for neurosyphilis, and it is administered by continuous intravenous, intermittent, or intramuscular injection. It is distributed in most tissues and body fluids, including lung, liver, bone, kidney, muscle, sputum, bile, urine and peritoneal, pleural and synovial fluid. It also penetrates the inflamed meninges and reaches therapeutic levels in the CSF, which is helpful in the management of neurosyphilis (12).

Globally, there are a significant number of women who do not attend prenatal care early, who only go to health services for childbirth care, or who, due to deficiencies in access or quality of care services, are not detected promptly with gestational syphilis. Therefore, there is reasonable doubt about what the literature says about the most effective antibiotic treatment regimen (in terms of dose, cycle length and mode of administration) for congenital syphilis in the last 12 weeks before delivery in women diagnosed with gestational syphilis. If the treatment is effective, it will not justify waiting until birth to start treatment, which benefits both the mother and the foetus and the new-born, in addition to reducing the costs for the treatment of the new-born and avoiding sequelae that affect the quality of life of the patient diagnosed with congenital syphilis.

Based on the above, this review will evaluate the evidence that supports different management alternatives, including benzathil penicillin cephalosporins, macrolides, and other types of penicillin, used in the last 12 weeks of pregnancy to treat GS and to prevent or treat CS and its complication

### **Research question:**

What is the safest and most effective antibiotic treatment regimen for congenital syphilis in the last

twelve weeks before delivery in women diagnosed with gestational syphilis?

### Primary objective

To evaluate the safety and effectiveness of the different treatment alternatives for congenital syphilis with untreated gestational syphilis or with suspected reinfection in the third trimester

**Secondary objectives:** To determine the safety and effectiveness of the different treatment regimens for gestational syphilis for the prevention of congenital syphilis by gestational age and time

### Methodology

Design: Systematic review of interventions

Criteria for considering studies for this review

**Types of studies:** We plan to include controlled clinical trials and case-control trials in this review. In case of not finding them, quasi-experimental studies, cohort studies, and case series will be included

**Type of population:** Participants of interest will be patients with a diagnosis confirmed gestational syphilis with a pregnancy of 28 weeks or more or patients who have suspected reinfection

**Type of intervention**

We will include studies that compare antibiotic treatment with any type of penicillin and alternative treatments, we will include all administration routes, doses and regimens without limits

**Exclusion criteria.** Studies where it is not possible to identify a separate way the women who receive treatment for GS in the third trimester

**Type of outcome measures.** Primary outcomes:

- Effectiveness: Maternal: Treatment failure for gestational syphilis. Treatment failure for congenital syphilis: evidenced by clinical signs, serological or cerebrospinal fluid titers equal to or greater than 4 times those of the mother and increased postnatal titers.
- Safety; ○ Presence of serious and non-serious adverse events related to the drug (systemic allergic reactions), Jarisch-Herxheimer reaction in the mother. ○ Adverse events in the foetus.

### Search Strategies

The following databases will be searched from 1990 to date, obtaining the results according to the term and the Boolean operator used: Cochrane Central Register of Controlled Trials, PUBMED, EMBASE. Other resources: We will check the bibliographies of the included studies and any relevant systematic reviews identified for additional references to relevant studies. We will contact experts and organizations to obtain additional information on relevant studies. If necessary, we will contact the authors of the included studies to clarify the data and obtain more information. There are no limits regarding the time of publication; Languages only publishes studies in English and Spanish.

### Selection of studies

Titles and abstracts obtained by electronic search will be downloaded from a reference management database, and duplicates will be removed. Two reviewers (VM AG) will independently screen titles and abstracts for inclusion. We will retrieve full-text reports and publications, then independently screen the complete text, identify studies for inclusion and identify and record reasons for exclusion of ineligible studies. We will resolve any disagreements by discussion or, if necessary, consult a third review author (HG). We will list studies that initially appeared to meet the inclusion criteria but were later excluded in the characteristics of excluded studies table.

### Data extraction and management

We will use a standard data collection form to study the characteristics and outcome data. Two reviewers (VM and AG) will independently extract characteristics of the included studies that include Methods: study design, number of study centres and location, study setting, withdrawals, study date, follow-up, and method of adjustment for design effects or confounding. Participants: number, mean age, parity, inclusion criteria, exclusion criteria, and other relevant characteristics. Intervention: dose, route, regimen, co-interventions, comparison and efficacy and safety primary or secondary outcomes.

Two reviewers (VM, AG) will independently extract outcome data from included studies. If outcome data were reported unusable, this would be indicated in the characteristics of the included studies table. Disagreements will be resolved by consensus or by involving a third reviewer (HG).

#### **Risk of bias assessment in included studies**

Two reviewers (VM, AG) will independently assess the risk of bias for key outcomes defined in this protocol using the Rob 2 tool for randomised studies and Robins for non-randomised studies. We will use the Newcastle Ottawa tool for cohort studies and the JBI Evidence Synthesis critical appraisal tool for case series. We will resolve disagreements by discussing or involving another author (HG).

#### **Treatment effect measures**

Data synthesis: This will be done narratively regarding the type of design, population, type of population included, intervention and risk of bias according to the kind of design. Unit of analysis: live births, stillbirths Effect measures: relative risk (RR) or odds ratio (OR) difference in cumulative incidence (risk difference)

Subgroup analysis: Subgroup analysis will be done according to gestational age of treatment between 28 and 31 6/7 weeks 32 to 35 6/7 and 36 or more weeks. The number of doses administered to the mother of the study drug and the diagnosed foetal compromise evidenced by ultrasound will also be analysed. A population subgroup will be analysed, including 4, 8 and 12 weeks before delivery.

**Presentation of results:** It will be done descriptively by type of design. The possibility of making a pooled estimate of the effect will be evaluated if the mixture of treatments or populations makes sense and depending on the heterogeneity of the results by type of design.

#### **Ethical Considerations**

This is a systematic review study without any intervention. The study will consider the ethical considerations of Resolution No. 8430 of 1993 of the Ministry of Health, which establishes the scientific, technical and administrative standards for research on humans. Article 11 of this resolution establishes the risks of research on humans, and according

to this classification, this project is classified as "Research without risk", which includes retrospective documentary research techniques and methods and those in which no intentional intervention or modification of the biological, physiological, psychological or social variables of the individuals participating in the study is carried out. The results obtained at the end of the study will be used solely for scientific purposes

## **BIBLIOGRAPHY**

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## APPENDIX 2. SEARCH STRATEGIES

Search electronic report #1	
Database	Ovid MEDLINE ALL(R) / PubMed(R) 1946 to Present
Platform	Ovid
Search Date	30/Jun/2025
Range of search date	None
Language restrictions	None
Other limits	None
Search strategy (results)	1 exp Syphilis/ (30643) 2 exp Syphilis, Latent/ (643) 3 exp Syphilis, Congenital/ (3209) 4 exp Syphilis Serodiagnosis/ (5978) 5 exp Treponema pallidum/ (4763) 6 syphilis.tw. (30421) 7 lues.tw. (509) 8 (treponema adj1 pallidum).tw. (5318) 9 (great adj1 pox).tw. (12) 10 (hutchinson\$ adj1 teeth).tw. (14) 11 or/1-10 (44190) 12 exp Pregnancy/ (1062441) 13 exp Pregnant People/ (16844) 14 pregnan\$.tw. (647967) 15 gestat\$.tw. (272371) 16 gravid\$.tw. (17812) 17 or/12-16 (1288526) 18 11 and 17 (4467) 19 exp Penicillins/ (87157) 20 exp Penicillin G/ (42901) 21 exp Penicillin G Benzathine/ (2043) 22 penicil?in\$.tw. (64356) 23 benzylpenicillin\$.tw. (2727) 24 benzathine.tw. (1301) 25 ampicillin.tw. (28999) 26 exp Cephalosporins/ (48534) 27 cephalospor\$.tw. (28270) 28 exp Cefotaxime/ (13574) 29 cephotaxim\$.tw. (67) 30 cefotaxim\$.tw. (10580) 31 exp Ceftriaxone/ (7229) 32 ceftriaxon*.tw. (15302) 33 exp Macrolides/ (126911) 34 macrolide\$.tw. (20712) 35 exp Erythromycin/ (28966) 36 erythromycin\$.tw. (24528) 37 exp Azithromycin/ (7361) 38 azithromycin\$.tw. (13160) 39 exp Clarithromycin/ (7329) 40 clarithromycin.tw. (11416) 41 exp Probenecid/ (3637) 42 probenecid.tw. (4042) 43 or/19-42 (329724) 44 18 and 43 (716)
# of records identified	716
# of records without duplicates	457

Search electronic report #2	
Database	EMBASE
Platform	Embase.com
Search Date	30/Jun/2025
Range of search date	None
Language restrictions	None
Other limits	None
Search strategy (results)	1 'syphilis'/exp (52862) 2 'latent syphilis'/exp (529) 3 'congenital syphilis'/exp (4550) 4 'syphilis serology'/exp (7193) 5 'treponema pallidum'/exp (8927) 6 syphilis:ti,ab (37531) 7 lues:ti,ab (786) 8 (treponema NEAR/1 pallidum):ti,ab (6666) 9 (great NEAR/1 pox):ti,ab (12) 10 (hutchinson* NEAR/1 teeth):ti,ab (4) 11 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 (62516) 12 'pregnancy'/exp (962156) 13 'pregnant person'/exp (139955) 14 pregnan*:ti,ab (903877) 15 gestat*:ti,ab (392598) 16 gravid*:ti,ab (25148) 17 #12 OR #13 OR #14 OR #15 OR #16 (1430225) 18 #11 AND #17 (6174) 19 'penicillin derivative'/exp (415095) 20 'penicillin g'/exp (87283) 21 'benzathine'/exp (267) 22 'procaine penicillin'/exp (5330) 23 penicil?in*:ti,ab (78593) 24 benzylpenicillin*:ti,ab (3235) 25 benzathine:ti,ab (1976) 26 'ampicillin'/exp (113496) 27 ampicillin:ti,ab (37467) 28 'cephalosporin derivative'/exp (316740) 29 cephalospor*:ti,ab (39504) 30 'cefotaxime'/exp (54722) 31 cephotaxim*:ti,ab (110) 32 cefotaxim*:ti,ab (14475) 33 'ceftriaxone'/exp (91391) 34 ceftriaxon*:ti,ab (26407) 35 'macrolide'/exp (469232) 36 macrolide*:ti,ab (27461) 37 'erythromycin'/exp (91419) 38 erythromycin*:ti,ab (30113) 39 'azithromycin'/exp (60641) 40 azithromycin*:ti,ab (21759) 41 'clarithromycin'/exp (46584) 42 clarithromycin:ti,ab (17648) 43 'probenecid'/exp (11513) 44 probenecid:ti,ab (5176) 45 #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25 OR #26 OR #27 OR #28 OR #29 OR #30 OR #31 OR #32 OR #33 OR #34 OR #35 OR #36 OR #37 OR #38 OR #39 OR #40 OR #41 OR #42 OR #43 OR #44 960358 46 #18 AND #45 1397 47 #18 AND #45 AND ((embase)/lim OR [preprint]/lim) (1121)
# of records identified	1121
# of records without duplicates	1110

Search electronic report #3	
Database	EBM Reviews - Cochrane Central Register of Controlled Trials
Platform	Ovid
Search Date	30/Jun/2025
Range of search date	None
Language restrictions	None
Other limits	None
Search strategy (results)	1 exp Syphilis/ (215) 2 exp Syphilis, Latent/ (5) 3 exp Syphilis, Congenital/ (13) 4 exp Syphilis Serodiagnosis/ (29) 5 exp Treponema pallidum/ (29) 6 syphilis.tw. (832) 7 lues.tw. (6) 8 (treponema adj1 pallidum).tw. (89) 9 (great adj1 pox).tw. (0) 10 (hutchinson\$ adj1 teeth).tw. (0) 11 or/1-10 (915) 12 exp Pregnancy/ (32926) 13 exp Pregnant People/ (0) 14 pregnan\$.tw. (75758) 15 gestat\$.tw. (31937) 16 gravid\$.tw. (1243) 17 or/12-16 (101087) 18 11 and 17 (194) 19 exp Penicillins/ (6704) 20 exp Penicillin G/ (5509) 21 exp Penicillin G Benzathine/ (140) 22 penicil?in\$.tw. (2591) 23 benzylpenicillin\$.tw. (117) 24 benzathine.tw. (183) 25 ampicillin.tw. (1556) 26 exp Cephalosporins/ (5156) 27 cephalospor\$.tw. (1542) 28 exp Cefotaxime/ (1652) 29 cephotaxim\$.tw. (2) 30 cefotaxim\$.tw. (786) 31 exp Ceftriaxone/ (855) 32 ceftriaxon*.tw. (1706) 33 exp Macrolides/ (11914) 34 macrolide\$.tw. (1210) 35 exp Erythromycin/ (4282) 36 erythromycin\$.tw. (1863) 37 exp Azithromycin/ (1525) 38 azithromycin\$.tw. (3355) 39 exp Clarithromycin/ (1741) 40 clarithromycin.tw. (3431) 41 exp Probenecid/ (314) 42 probenecid.tw. (489) 43 or/19-42 (29078) 1 44 18 and 43 (38)
# of records identified	38
# of records without duplicates	19

## APPENDIX 3. RISK OF BIAS IN COHORT STUDIES WITHOUT CONTROL GROUP. GETS TOOL

Checklist for series of subjects exposed to intervention studies			
Reference:			
Checklist verified by:			
<b>Section 1 Internal validity</b>			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
Selection	Does the study define an appropriate, clear, and focused research question?	Yes	
		No	
		Unclear	
Selection			
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention?	Yes	
		No	
		Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention?	Yes	
		No	
		Unclear	
1.4	Is clear the proportion of eligible subjects who participated in the study? (Describe the proportion, please )	Yes	
		No	
		Unclear	
1.5	Were exposed subjects included in strict sequential order?	Yes	
		No	
		Unclear	
Measurement			
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention?	Yes	
		No	
		Unclear	
1.7	Is the intervention applied to the subjects clearly defined?	Yes	
		No	
		Unclear	
1.8	Is the level to exposition to intervention described in detail (adherence measurement)?	Yes	
		No	
		Unclear	

1.9	Were any co-interventions applied differentially to the exposed subjects?	Yes	
		No	
		Unclear	
1,10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?	Yes	
		No	
		Unclear	
1.11	Are prognostic variables adequately defined and measured?	Yes	
		No	
		Unclear	
1.12	Are outcome variables adequately defined and measured?	Yes	
		No	
		Unclear	
<b>Attrition</b>			
1.13	Is there a description of proportion of subjects were lost to follow-up?	Yes	
		No	
		Unclear	
<b>Confounding</b>			
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?	Yes	
		NO	
		Unclear	
<b>Other problems</b>			
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention?	Yes	
		NO	
		Unclear	
1.16	Are confidence intervals for the absolute effect of the intervention presented	Yes	
		NO	
		Unclear	
<b>Section 2 – Overall Evaluation of the Study</b>			
2,1	Considering clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	
		NO	
		Unclear	
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	
		NO	
		Unclear	

GETS: Grupo de Evaluación de Tecnologías y Políticas en Salud.

Assessment instruction

### **Risk of Bias Assessment**

#### ***Selection Bias (Item 1.2 -1.5)***

- Low: All of items 1.2 to 1.5 are responded Yes
- Moderate: Item 1.3 is responded No
- High: Items 1.2, 1.4, or 1.5 are responded No
- Unclear: Two or more items are unclear

#### ***Performance and Detection Bias (Item 1.6 - 1.12)***

- Low: All of items 1.6 to 1.12 are responded Yes
- Moderate: One item responded No
- High: Two or more items are responded No
- Unclear: Two or more items are unclear

#### ***Attrition Bias (Item 1.13)***

- Low: Item 1.13 is responded Yes
- High: The item is responded No
- Unclear: The item is unclear

#### ***Confounding Bias (Item 1.14)***

- Moderate: Robust methodologies (e.g., causal inference: time series, segmented regression, before and after study assuming no independence, etc.) are used to control confounding factors
- High: Descriptive post-intervention measures or pre-post comparisons assuming independence are used

#### ***Other Issues (Items 1.15–1.16)***

- Low: Both items are responded Yes
- Moderate: One item is responded No
- High: Both items are Responded No
- Unclear: Both items are unclear

#### ***Overall Risk of Bias***

- Low Risk: All domains have low risk of bias and no other relevant issues
- High Risk: One domain has high risk of bias and no other issues
- Very High Risk: Two or more domains have high risk of bias, or one domain with high risk and additional relevant issues

## APPENDIX 4. LIST OF EXCLUDED REFERENCES

Reference	Reason for exclusion
Alberto C, Wagner N, Fougère Y, Meyer Sauteur PM, Scherler G, Aebbi-Popp K, et al. Syphilis in pregnant women and congenital syphilis from 2012 to 2021 in Switzerland: a multicentre, retrospective study. <i>Swiss Medical Weekly</i> . 2024;154(11).	No 3rd trimester data available
Gao J, Chen X, Yang M, Wu Y, Liang T, Li H, et al. Adverse pregnancy outcomes and associated risk factors among pregnant women with syphilis during 2013–2018 in Hunan, China. <i>Frontiers in Medicine</i> . 2023;10.	No 3rd trimester data available
Iroha K, Masashi D, Kenji T, Yutoku S, Akiko U, Tokuro S, et al. Case report of fourteen pregnant women with syphilis. <i>Journal of Obstetrics and Gynaecology Research</i> . 2023;49(1):447.	Full text not retrieved
Johnson KA, Burghardt NO, Snyder RE, Plotzker RE, Imp BM, Murphy R, et al. Comparing 7-Day Versus 6–8-Day Penicillin Treatment Intervals Among Pregnant People With Syphilis of Late or Unknown Duration: No Difference Found in Incidence of Congenital Syphilis. <i>Open Forum Infectious Diseases</i> . 2023;10(6).	No 3rd trimester data available
Liu H, Chen N, Tang W, Shen S, Yu J, Xiao H, et al. Factors influencing treatment status of syphilis among pregnant women: a retrospective cohort study in Guangzhou, China. <i>International Journal for Equity in Health</i> . 2023;22(1).	No 3rd trimester data available
Mo XF, Feng MZ, Jiang TT, Xu YT, Wu MJ, Li JJ, et al. Treatment of maternal syphilis for preventing congenital syphilis: an observational study of adherence to treatment recommendation in Suzhou, China, 2019–2021. <i>Sexual Health</i> . 2023;20(6):523–30.	Full text not retrieved
Patil S, Li X, Liu P, Dai Y, Zhu X, Li J, et al. A Retrospective Cohort Study on <i>Treponema pallidum</i> Infection: Clinical Trends and Treatment Outcomes for Congenital Syphilis in Guangxi, China (2013–2023). <i>Infection and Drug Resistance</i> . 2024;17:2351–9.	No 3rd trimester data available
Sangtawesin V, Lertsutthiwong W, Kanjanapattanakul W, Khorana M, Horpaopan S. Outcome of maternal syphilis at Rajavithi Hospital on offsprings. <i>Journal of the Medical Association of Thailand</i> . 2005;88(11):1519–25.	(“Randomized C
Sayal HB, Yavuz A, Tsakir B, Toprak E, Han O, Inal H. Maternal and neonatal outcomes of congenital syphilis at a tertiary care center in Turkey; a retrospective observational study. <i>Journal of Obstetrics and Gynaecology</i> . 2024;44(1).	No 3rd trimester data available
Tannis A, Miele K, Carlson JM, O’Callaghan KP, Woodworth KR, Anderson B, et al. Syphilis Treatment among People Who Are Pregnant in Six U.S. States, 2018–2021. <i>Obstetrics and Gynecology</i> . 2024;143(6):718–29.	No 3rd trimester data available
Wang H, Ying X, Lin D, Uwimana MMP, Zhang X. Towards the elimination of mother to child transmission of syphilis 2015–2020: practice and progress in Zhejiang province, eastern China. <i>BMC Pregnancy and Childbirth</i> . 2023;23(1).	No 3rd trimester data available

## APPENDIX 5. RISK OF BIAS ASSESSMENT OF INCLUDED STUDIES

Checklist for series of subjects exposed to intervention studies			
Reference: Alexander JM, Sheffield JS, Sanchez PJ, Mayfield J, Wendel GD Jr. Efficacy of treatment for syphilis in pregnancy. <i>Obstet Gynecol.</i> 1999 Jan;93(1):5-8. doi: 10.1016/s0029-7844(98)00338-x			
Checklist verified by: HGD, AMGT			
<b>Section 1 Internal validity</b>			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
1.1	Does the study define an appropriate, clear, and focused research question?	Yes	X
	“To evaluate the efficacy of the CDC recommendations for the treatment of syphilis in pregnancy for the eradication of maternal infection and prevention of congenital syphilis.”	No	
		Unclear	
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention?	Yes	X
	“Rapid plasma reagin test, VDRL test with titer and confirmatory microhemagglutinin assay for antibodies to <i>Treponema pallidum</i> were used to identify infected patients.”	No	
		Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention?	Yes	
	There is no information	No	
		Unclear	X
1.4	What proportion of eligible subjects participated in the study?	Yes	X
	340/340 (100%)	No	
		Unclear	
1.5	Were exposed subjects included in strict sequential order?	Yes	
	It looks like yes	No	
		Unclear	X
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention?	Yes	
	There is not information	No	X
		Unclear	
1.7	Is the intervention applied to the subjects clearly defined?	Yes	X
	Four hundred forty-eight women (1.6% of the population) had untreated syphilis, of those 340 (75.9%) received prenatal care and antepartum therapy”	No	
		Unclear	

1.8	Is the intervention described in sufficient detail to allow replication (definition and components)? Primary, secondary or early latent (under 1 year duration) syphilis was treated with 2,400,000 units intramuscular (IM) benzathine penicillin G. Late latent syphilis (uncertain or longer than 1 year duration) was treated with 7,200,000 units IM benzathine penicillin G over 3 consecutive weeks	Yes	<b>X</b>
		No	
		Unclear	
1.9	Were any co-interventions applied differentially to the exposed subjects?  There is not information	Properly treated	
		Unproperly treated	
		It is not clear	<b>X</b>
1.10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?  Open-label study. Hard outcome. Measured with non-treponemal test titers for the mother and complete serological and clinical examination of the new-born and isolation of confirmed tp.	Yes	
		No	<b>X</b>
		Unclear	
1.11	Are prognostic variables adequately defined and measured?  There is not information	Yes	
		No	<b>X</b>
		Unclear	
1.12	Are outcome variables adequately defined and measured?  For GS. Clinical cure was defined by the resolution of symptoms in patients with primary or secondary syphilis and a fourfold decrease in titer over $3 \pm 4$ months. In patients with latent syphilis, a fourfold decrease in titer over 6 months was considered a clinical cure. Treatment failure was defined as a persistent (longer than 1 month) fourfold increase in titer, or an unchanged titer 6 months after treatment.  For CS, the diagnosis of congenital syphilis was based on the criteria established by Kaufmann. <sup>6,13</sup> A definite case of congenital infection required identifying <i>T pallidum</i> specimens from lesions by darkfield microscopy or histologic examination. Probable case of congenital syphilis required clinical manifestation of disease, along with a confirmatory microhemagglutinen assay for antibodies to <i>T pallidum</i> , a rising VDRL over the first 3 months of life, or a persistent VDRL over the first 4 months of life.	Yes	<b>X</b>
		No	
		Unclear	

1.13	What proportion of subjects were lost to follow-up?	Yes	
		No	
	There is not information about time of follow up	Unclear	<b>X</b>
<b>Section 2 – Overall Evaluation of the Study</b>			
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?	Yes	
		No	<b>X</b>
	No for gestational age time to deliver or re-infected. Authors do not use robust analysis for causal inference	Unclear	
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention?	Yes	
		No	<b>X</b>
	No	Unclear	
1.16	Are confidence intervals for the absolute effect of the intervention presented	Yes	<b>X</b>
		No	
		Unclear	
<b>Section 2 – Overall Evaluation of the Study</b>			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	<b>X</b>
		No	
		Unclear	
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	<b>X</b>
		No	
		Unclear	

Risk of Bias Assessment

Risk of selection bias “Unclear”

Performance and detection Bias: High (1.10, 1.11 Inadequate treated)

Incomplete data Bias: Unclear

Confusion bias: High

Other problems: Yes

Overall; High Risk

Checklist for series of subjects exposed to intervention studies			
Reference: Dou L, Wang X, Wang F, Wang Q, Qiao Y, Su M, Jin X, Qiu J, Song L, Wang A. Epidemic Profile of Maternal Syphilis in China in 2013. Biomed Res Int. 2016;2016:9194805. doi: 10.1155/2016/9194805.			
Checklist verified by: HGD, AMGT			
Section 1 Internal Validity			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		This study is. (Mark with an X the selected answer)	
1.1	Does the study define an appropriate, clear, and focused research question? to describe the epidemiological characteristics of pregnant women with maternal syphilis and their pregnancy outcomes in China in 2013	Yes	
		No	
		Unclear	<b>X</b>
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention?  Pregnant women who met the following criteria and their children were included in the analysis: (1) syphilis screening tests positive during pregnancy and delivery at health facilities; (2) being diagnosed with syphilis; (3) being registered in the surveillance system; (4) delivered at gestational age of 28 weeks or more in 2013	Yes	<b>X</b>
		No	
		Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention?  Newborns with congenital syphilis born to women who received no syphilis tests were excluded from the analyses	Yes	<b>X</b>
		No	
		Unclear	
1.4	What proportion of eligible subjects participated in the study?  In 2013, a total of 15884 pregnant women with laboratory-confirmed syphilis infection delivered and were registered in Information System of Prevention of Mother to child transmission of syphilis management in China. All of them were considered to the analysis	Yes	<b>X</b>
		No	
		Unclear	
1.5	Were exposed subjects included in strict sequential order?  It looks like yes	Yes	<b>X</b>
		No	
		Unclear	

1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention?	Yes	X
		No	
	Tables 1, 2, 3	Unclear	
1.7	Is the intervention applied to the subjects clearly defined?	Yes	X
	For those with positive syphilis tests appropriate treatment will be initiated promptly with at least 2 courses of penicillin treatment, one at early pregnancy stage and one at the third trimester.	No	
	For each course women will receive either benzathine or procaine penicillin treatment by intramuscular injection, as recommended by the guide lines.	Unclear	
1.8	Is the intervention described in sufficient detail to allow replication (definition and components)?	Yes	
	Inadequate treatment for maternal syphilis was defined as nontreatment or nonpenicillin treatment, less than 2 completed courses of treatment during pregnancy, or less than 2 weeks between the 2 courses of treatment	No	X
	In the group of women with treatment in the last 4 weeks, it is not possible to identify who was treated and who was not treated or who was inadequately treated, so there is detection bias when mixing them.	Unclear	
1.9	Were any co-interventions applied differentially to the exposed subjects?	Yes	
		No	
	There is not information	Unclear	X
1,10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?	Yes	
		No	X
	Open label study. Hard outcomes	Unclear	
1.11	Are prognostic variables adequately defined and measured?	Yes	
	Gestational age is not defined	No	
	Time between treatment and delivery was not considered	Unclear	X

1.12	Are outcome variables adequately defined and measured? Congenital syphilis in new-born was defined as having at least one of the following situations in infant who was younger than 6 weeks and born to syphilis infected mother: (1) positive treponemal test and high titer of nontreponemal test (a value 4-fold higher than that of his/her mother's before delivery); (2) laboratory confirmation of <i>Treponema pallidum</i> in clinical specimens by dark-field microscopy; or (3) reactive treponemal antibody test of IgM.	Yes	<b>X</b>
	“Any adverse pregnancy outcomes” are a composite outcome in our analysis, which include preterm delivery, LBW, stillbirth, neonatal death, and congenital syphilis in newborns.	No	
	Preterm delivery was defined as a delivery that occurs before 37 gestational weeks. Low birth weight (LBW) was defined as birth weight of a live-born baby of less than 2,500g at birth. Stillbirth was defined as death of a foetus of at least 28 gestational weeks. Neonatal death was defined as death of live-born baby who died during the first week after birth.	Unclear	
1.13	What proportion of subjects were lost to follow-up?	Yes	
		No	<b>X</b>
	Apparently, there was not	Unclear	
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?	Yes	
		NO	<b>X</b>
	Authors do not describe loss of follow up	Unclear	
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention?	Yes	
		NO	<b>X</b>
		Unclear	
1.16	Are confidence intervals for the absolute effect of the intervention presented	Yes	<b>X</b>
		NO	
		Unclear	

Section 2 – Overall Evaluation of the Study			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	<b>X</b>
		NO	
		Unclear	
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	
		NO	
	Not for pregnancy of 37 weeks or more	Unclear	<b>X</b>

**Risk of Bias Assessment**

Risk of selection bias; low

Performance and detection Bias: High (1.8 Inadequate treated)

Incomplete data Bias: Unclear

Confusion bias: High

Other problems: Yes (no CI 95%)

Overall; Very High Risk

Checklist for series of subjects exposed to intervention studies			
Reference: Hong FC, Risk of Congenital Syphilis (CS) Following Treatment of Maternal Syphilis: Results of a CS Control Program in China. Clin Infect Dis. 2017 Aug 15;65(4):588-594. doi: 10.1093/cid/cix371			
Checklist verified by:			
Section 1 Internal validity			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
1.1	Does the study define an appropriate, clear, and focused research question?	Yes	<b>X</b>
	To evaluate the efficacy of the CDC recommendations for the treatment of syphilis in pregnancy for the eradication of maternal infection and prevention of congenital syphilis	No	
		Unclear	
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention?	Yes	<b>X</b>
	Cohorts of pregnant women were structured by data drawn from master datasets to include pregnant women who were identified with syphilis-seropositivity at their first antenatal visit and had information on their treatment status for syphilis. with no treatment before pregnancy.	No	
	The pregnant women finally included for analyses were limited to those women whose infants had a definite outcome of confirming or excluding a diagnosis of congenital syphilis by October 2016	Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention?	Yes	
	There is not information	No	
		Unclear	<b>X</b>

1.4	What proportion of eligible subjects participated in the study?	Yes	<b>X</b>
	7,498 had Syphilis 6,559 were included (88%) 4,746 were analysed (76% of those included)	No	
		Unclear	
1.5	Were exposed subjects included in strict sequential order?	Yes	
	It looks like	No	
		Unclear	<b>X</b>
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention? Tables 1, 3	Yes	<b>X</b>
		No	
		Unclear	
1.7	Is the intervention applied to the subjects clearly defined?	Yes	<b>X</b>
	Intramuscular benzathine penicillin G (BPG) was applied as the first choice to all syphilis-seropositive pregnant women regardless of the disease stage for at least one course (2.4 million units of BPG once weekly for three consecutive weeks). A second-course of treatment during late pregnancy was applied in the study area before it was recommended by the national PTNCT guidelines in 2012. Procaine penicillin G (PPG) 0.8 million units intramuscularly once daily for 15 days was introduced during 2003-2008. Pregnant women who were allergic to penicillin were treated with erythromycin 500 mg orally four times a day for 15 days, azithromycin 500mg orally once a day for 10 days, or ceftriaxone sodium 1 g injection per day for 10 days	No	
		Unclear	
1.8	Is the intervention described in sufficient detail to allow replication (definition and components)?	Yes	
	Based on treatment status prior to the current pregnancy, the syphilis-seropositive pregnant women were categorized into Group 1 with adequate treatment before pregnancy and Group 2 with no treatment before pregnancy. Each of the groups was further grouped into (1) treatment with early BPG (initiation of BPG or PPG before 28 weeks of gestation), (2) treatment with late BPG (initiation of BPG or PPG at 28 weeks of gestation or later), (3) treatment with other antibiotics and (4) no treatment during pregnancy	No	
		Unclear	
1.9	Were any co-interventions applied differentially to the exposed subjects?	Yes	
	There is not information	No	<b>X</b>
		Unclear	
1,10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?	Yes	
	Open label study Hard outcome for CS	No	<b>X</b>
		Unclear	

1.11	Are prognostic variables adequately defined and measured?	Yes	
		No	
	Gestational age No time between Treatment and delivery	Unclear	<b>X</b>
1.12	Are outcome variables adequately defined and measured? CS Diagnosis of 4 congenital syphilis (probable or confirmed case) was based on meeting one of the following laboratory criteria: (1) a reactive 19S-IgM-TPPA; (2) a serum quantitative TRUST titer that was four-fold higher than the mother's titer; (3) a four-fold or greater rise in TRUST titer during the follow-up; or (4) a reactive TPPA that did not revert to nonreactive by end of 18 months' follow-up. Stillbirth potentially due to syphilis was not accounted into cases of congenital syphilis.  GS: 3 months to monitor any changes in TRUST titer and TPPA positivity.	Yes	<b>X</b>
		No	
		Unclear	
1.13	What proportion of subjects were lost to follow-up? Excluding 889 (11.9%) women who were lost to follow-up Of these infants, 27.9% were lost from the cohort mainly because of migration of their parents (66.9%)	Yes	<b>X</b>
		No	
		Unclear	
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?	Properly treated	
		Unproperly treated	<b>X</b>
		Not clear	
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention? No, they include all participants	Yes	
		No	<b>X</b>
		Unclear	
1.16	Are confidence intervals for the absolute effect of the intervention presented	Yes	<b>X</b>
		No	
	CI 95 it is presented	Unclear	
Section 2 – Overall Evaluation of the Study			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	<b>X</b>
		No	
		Unclear	
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	<b>X</b>
		No	
		Unclear	

Risk of Bias Assessment

Risk of selection bias “High”

Performance and detection Bias: Low (1.10, 1.11 Inadequate treated)

Incomplete data Bias: High

Confusion bias: High

Other problems: Yes

Overall; Very High Risk

Checklist for series of subjects exposed to intervention studies			
Reference: Luengmettakul J, Apiwintana S, Jitrungruengnij N. Incidence of Congenital Syphilis and Adverse Pregnancy Outcomes among Syphilitic Pregnant Women According to the Treatment Adequacy in Tertiary Care Hospital, Thailand. J Med Assoc Thai 2025;108:9-16. DOI: 10.35755/jmedassocthai.2025.1.9-16-01174			
Checklist verified by: HGD, AMGT			
<b>Section 1 Internal validity</b>			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
1.1	Does the study define an appropriate, clear, and focused research question? “Study the association of congenital syphilis and adverse pregnancy outcomes between mothers who received adequate treatment and those who received inadequate treatment”.	Yes	<b>X</b>
		No	
		Unclear	
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention? “The authors consecutively collected the medical records of pregnant women diagnosed with syphilis during pregnancy, provided there were no missing important data, until the specified number was reached”	Yes	<b>X</b>
		No	
		Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention? No information	Yes	
		No	
		Unclear	<b>X</b>
1.4	What proportion of eligible subjects participated in the study? 106 of 162 with adequate treatment. 90 with inadequate treatment and 16 without treatment. 65% of adequate treatment 100 % of inadequate treatment + 100% untreated	Yes	<b>X</b>
		No	
		Unclear	
1.5	Were exposed subjects included in strict sequential order? Authors don't say how they selected the 106 with adequate treatment.	Yes	
		No	
		Unclear	<b>X</b>
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention? See at Table 1	Yes	
		No	
		Unclear	
1.7	Is the intervention applied to the subjects clearly defined? “Adequate treatment for syphilis infection during pregnancy means receiving a complete course of Penicillin injection at least 30 days before delivery. Inadequate treatment is defined as receiving a Complete course of penicillin but less than 30 days before delivery, or receiving an incomplete injection, or not receiving treatment during pregnancy. Mixed inadequately treated with untreated”	Yes	
		No	<b>X</b>
		Unclear	

1.8	Is the intervention described in sufficient detail to allow replication (definition and components, mode of administration)?  “The authors treat early syphilis with benzathine penicillin intramuscularly (IM) for one or two doses. The late syphilis was treated with benzathine penicillin IM weekly for three doses Pregnant women allergic to penicillin might use penicillin sensitization or alternative medications, depending on the patient’s preference”.	Yes	X
		No	
		Unclear	
1.9	Were any co-interventions applied differentially to the exposed subjects?  There is not information	Yes	
		No	X
		Unclear	
1,10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?  The inadequate treatment and not treatment are included in the same group it could carry on to overestimation of CS incidence. In 30% of patients with adequate treatment we do not have data	Yes	
		No	X
		Unclear	
1.11	Are prognostic variables adequately defined and measured?  There is not information	Yes	
		No	
		Unclear	X
1.12	Are outcome variables adequately defined and measured? “Congenital syphilis is defined by the Centres for Disease Control and Prevention (CDC)(15) as 1) a neonate or foetus with an abnormal physical examination that was consistent with congenital syphilis, 2) a serum nontreponemal serologic titer that was fourfold higher than the mother’s titer, or 3) a pathological placenta confirmed for syphilis infection.	Yes	X
		No	
		Unclear	
1.13	What proportion of subjects were lost to follow-up?  Apparently, they had no losses	Properly treated	
		Unproperly clear	
		Unclear	
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?  Authors do not take into account the time from treatment to delivery or gestational age. No robust statistical methods are used to control for confounding factors.	Yes	
		No	X
		Unclear	
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention? No, they don’t	Yes	
		No	X
		Unclear	

1.16	Are confidence intervals for the absolute effect of the intervention presented	Yes	<b>X</b>
		No	
		Unclear	
<b>Section 2 – Overall Evaluation of the Study</b>			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	
		No	
		Unclear	<b>X</b>
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	<b>X</b>
		No	
		Unclear	

**Risk of Bias Assessment**

Risk of selection bias “High”

Performance and detection Bias: High

Incomplete data Bias: Low

Confusion bias: High

Other problems: Yes

Overall; Very High Risk

**Checklist for series of subjects exposed to intervention studies**

Reference: Nishijima Effectiveness and Tolerability of Oral Amoxicillin in Pregnant Women with Active Syphilis, Japan, 2010-2018. Emerg Infect Dis. 2020 Jun;26(6):1192-1200. doi: 10.3201/eid2606.191300

Checklist verified by: HGD, AMGT

**Section 1 Internal validity**

In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
1.1	Does the study define an appropriate, clear, and focused research question?	Yes	<b>X</b>
	To evaluate the efficacy of the CDC recommendations for the treatment of syphilis in pregnancy for the eradication of maternal infection and prevention of congenital syphilis	No	
		Unclear	
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention?	Yes	<b>X</b>
	We included pregnant women with syphilis, regardless of their symptoms, who were treated with oral amoxicillin or ampicillin during 2010–2018; we only included those with serum rapid plasma reagin (RPR) titers >8 and positive results from treponemal tests, such as the T. pallidum hemagglutination test (10,15)	No	
		Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention?	Yes	<b>X</b>
	We excluded patients with tertiary syphilis or neurosyphilis diagnoses that were based on findings from cerebrospinal fluid (CSF) samples (10,16) for patient symptoms (including ocular or auditory syphilis) and those with suspected reinfections after initiation of syphilis treatment (defined as patients with >4-fold rise in RPR titer after 4-fold decrement with or without symptoms) (10,15,17).	No	
		Unclear	

1.4	What proportion of eligible subjects participated in the study? 80 of 136 (60%) were admitted (Authors do not give reasons for exclusion)	Yes	
		No	
		Unclear	X
1.5	Were exposed subjects included in strict sequential order?  There is not information	Yes	
		No	
		Unclear	X
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention? Table 1	Properly treated	X
		Unproperly treated	
		Unclear	
1.7	Is the intervention applied to the subjects clearly defined?  These guidelines recommend oral amoxicillin or ampicillin with dosing of 1,500 mg/day (i.e., 500 mg 3 X/d) for 2–4 weeks for primary syphilis, 4–8 weeks for secondary syphilis, and 8–12 weeks for tertiary or later-stage syphilis in pregnant women.	Yes	X
		No	
		Unclear	
1.8	Is the intervention described in sufficient detail to allow replication (definition and components)?  Yes	Yes	X
		No	
		Unclear	
1.9	Were any co-interventions applied differentially to the exposed subjects? There is not information	Yes	
		No	
		Unclear	X
1,10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?  Open-label study. Hard outcome. Measured with non-treponemal test titers for the mother and serologic examination, and a complete five-titer test for the new-born (probable) and isolation of confirmed tp.	Yes	
		No	X
		Unclear	
1.11	Are prognostic variables adequately defined and measured? Table 1	Yes	X
		No	
		Unclear	
1.12	Are outcome variables adequately defined and measured?  The primary outcome of this study was effectiveness of prevention of MTCT of syphilis. We defined MTCT as a CS case and defined CS cases as live newborns with CS diagnoses, miscarriages, or still births. If a live new-born was not given a CS diagnosis, we interpreted the treatment given as successful for preventing MTCT (5). We evaluated the secondary outcome serologic effectiveness of treatment in the mother (4-fold decrement of serum RPR titer) at each of the following time points: delivery (25), 6 months after therapy (4), and 12 months after. Not in the least 4 weeks	Yes	X
		No	
		Unclear	

1.13	What proportion of subjects were lost to follow-up?	Yes	X
	Of the 80 cases, we excluded 9 (6 with unknown outcomes, 3 involving abortions induced at 15–17weeks of pregnancy) from the outcome analysis.	No	
		Unclear	
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?	Yes	
	Not reinfection; it appears to have been assumed to be treatment failure. They did not use robust methods to evaluate causal inference.	No	X
		Unclear	
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention?	Yes	
	No	No	X
		Unclear	
1.16	Are confidence intervals for the absolute effect of the intervention presented	Yes	X
	CI are presented	No	
		Unclear	
<b>Section 2 – Overall Evaluation of the Study</b>			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	X
		No	
		Unclear	
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	X
		No	
		Unclear	

Risk of Bias Assessment

Risk of selection bias “High” (1.4)

Performance and detection Bias: “Low

Incomplete data Bias: “Low

Confusion bias: “High”

Other problems: Yes

Overall; Very High Risk

Checklist for series of subjects exposed to intervention studies			
Reference: Qin JB, Feng TJ, Yang TB, Hong FC, Lan LN, Zhang CL. Maternal and paternal factors associated with congenital syphilis in Shenzhen, China: a prospective cohort study. Eur J Clin Microbiol Infect Dis. 2014 Feb;33(2):221-32. doi: 10.1007/s10096-013-1948-z.			
Checklist verified by:			
<b>Section 1 Internal validity</b>			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
1.1	Does the study define an appropriate, clear, and focused research question? The study aimed to investigate the maternal and paternal risk factors for CS.	Properly treated	X
		Unproperly treated	
		Unclear	
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention?  The inclusion criteria were women who: (a) had positive TRUST and TPPA tests during pregnancy or up to a week after delivery; (b) provided informed consent to be included in the evaluation; (c) continued their pregnancy after treatment; (d) participated in the follow-up process and had a complete case report form (CRF); (e) had a pregnancy outcome that could be clearly evaluated; (f) were not allergic to benzathine penicillin G; and (g) had a negative HIV test	Properly treated	X
		Unproperly treated	
		Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention?  HIV(+) spontaneous abortion or IVE, ectopic pregnancy, foetal death, allergy to penicillin. Incomplete data	Properly treated	
		Unproperly treated	X
		Unclear	
1.4	What proportion of eligible subjects participated in the study?  52%	Properly treated	
		Unproperly treated	X
		Unclear	
1.5	Were exposed subjects included in strict sequential order?  There is no information	Properly treated	
		Unproperly treated	
		Unclear	X
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention?  Table 1, 3, 4	Properly treated	
		Unproperly treated	
		Unclear	X
1.7	Is the intervention applied to the subjects clearly defined?  Gravidas with untreated syphilis were given three injections of 2.4 million units of benzathine penicillin at weekly intervals. At least one dose was given if there was too little time to give injections before delivery.	Properly treated	X
		Unproperly treated	
		Unclear	

1.8	Is the intervention described in sufficient detail to allow replication (definition and components)? Complete syphilis treatment was defined as three doses of benzathine penicillin at weekly intervals [21]. Incomplete treatment was defined as two doses or less of penicillin received.  This data was not taken into account to evaluate the Incidence of CS at GA > 29 week or four weeks before pregnancy	Properly treated	
		Unproperly treated	X
		Unclear	
1.9	Were any co-interventions applied differentially to the exposed subjects?  There is no information	Properly treated	
		Unproperly treated	
		Unclear	X
1,10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?  Hard Outcome see 1.12	Properly treated	X
		Unproperly treated	
		Unclear	
1.11	Are prognostic variables adequately defined and measured?  No time to delivery	Properly treated	X
		Unproperly treated	
		Unclear	
1.12	Are outcome variables adequately defined and measured?  Diagnosis of congenital syphilis A CS diagnosis required one of the following criteria: (1) a positive dark field or fluorescent antibody test of body fluid; (2) an abnormal physical examination that was consistent with secondary syphilis and a TRUST titer that was four-fold higher than the mother's titer;(3) a positive fluorescent treponemal antibody absorption test with a fluorochrome-labelled antihuman IgM on fractionated sera (FTA-ABS19SIgMtest); or (4) a reactive TPPA test until 18 months after birth [16, 22	Properly treated	X
		Unproperly treated	
		Unclear	
1.13	What proportion of subjects were lost to follow-up?  Losses in the follow-up 32% In newborns, there was no measure of the effect in breasts	Properly treated	
		Unproperly treated	X
		Unclear	
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?  Authors did not use causal inference for analysis	Properly treated	
		Unproperly treated	X
		Unclear	
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention?	Properly treated	
		Unproperly treated	
		Unclear	X

1.16	Are confidence intervals for the absolute effect of the intervention presented	Properly treated	
		Unproperly treated	X
		Unclear	
<b>Section 2 – Overall Evaluation of the Study</b>			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	
		No	
		Unclear	X
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	X
		No	
		Unclear	

**Risk of Bias Assessment**

Assessment

Selection Bias: high

Performance and detection bias:

Attrition Bias

Confusion Bias

Other problems

Overall Risk

<b>Checklist for series of subjects exposed to intervention studies</b>			
Reference: Wan Z, Zhang H, Xu H, Hu Y, Tan C, Tao Y. Maternal syphilis treatment and pregnancy outcomes: a retrospective study in Jiangxi Province, China. BMC Pregnancy Childbirth. 2020 Oct 27;20(1):648. doi: 10.1186/s12884-020-03314-y			
Checklist verified by:			
<b>Section 1 Internal validity</b>			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
1.1	Does the study define an appropriate, clear, and focused research question?  The purpose of this study was to investigate the impact of maternal treatment during pregnancy-on-pregnancy outcomes in Jiangxi province.	Yes	X
		No	
		Unclear	
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention?  Given that data which included only syphilis infected pregnant women who delivered at gestational age of 28weeks or more (including live births, stillbirth $\geq$ 28 gestational weeks and 0–7 days for neonatal deaths) and were registered in Information System of PMTCT of Syphilis Management between 1 January 2013 and 31 December 2019 were enrolled	Yes	X
		No	
		Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention?  61 women were excluded from the study because of delivering multiple pregnancy (46) or lacking of treatment information	Yes	
		No	
		Unclear	X

1.4	What proportion of eligible subjects participated in the study?	Yes	<b>X</b>
	During 1 January 2013–31 December 2019, 3,945,102 pregnant women were screened for syphilis and 4271 who delivered at gestational age of 28 weeks or more and were registered in Information System of PMTCT of Syphilis Management of Jiangxi Province were enrolled. Among these women, 61 women (1.6%) were excluded from the study because of delivering multiple pregnancy (46) or lacking of treatment information (15), thus resulting in 4210 women included in the final analyses (Fig. 1).	No	
		Unclear	
1.5	Were exposed subjects included in strict sequential order? It looks like	Yes	<b>X</b>
		No	
		Unclear	
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention? Table 1	Yes	<b>X</b>
		No	
		Unclear	
1.7	Is the intervention applied to the subjects clearly defined? Author do not define clearly the intervention	Yes	
		No	
		Unclear	<b>X</b>
1.8	Is the intervention described in sufficient detail to allow replication (definition and components)?  Definitions Adequate treatment was defined as two completed courses of penicillin treatment with more than 2 weeks (appropriately 4 weeks) between the two courses, and treatment must have been provided at least 28 days prior to delivery or 4 weeks gestation. Non-penicillin treatment or treatment with fewer than two completed courses was defined as treated inadequately.	Yes	<b>X</b>
		No	
		Unclear	
1.9	Were any co-interventions applied differentially to the exposed subjects? There is not information	Yes	
		No	
		Unclear	<b>X</b>
1,10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment? There is not information	Yes	
		No	
		Unclear	<b>X</b>
1.11	Are prognostic variables adequately defined and measured? No data is provided on time between treatment and delivery cases of reinfection. Table 1 does provide information on the trimester of pregnancy at the start of CPN.	Yes	<b>X</b>
		No	
		Unclear	

1.12	Are outcome variables adequately defined and measured?	Yes	
	Congenital syphilis in new-born was defined as having at least one of the following situations in infant who was younger than 6 weeks and born to syphilis infected mother: (1) positive treponemal test and high titer of nontreponemal test (a value 4-fold higher than that of his/her mother's before delivery); (2) laboratory confirmation of Treponema pallidum in clinical specimens by dark-field microscopy. It does not describe separately how many patients in the third trimester received adequate or inadequate treatment	No	
		Unclear	<b>X</b>
1.13	What proportion of subjects were lost to follow-up?	Yes	<b>X</b>
	Lost to follow-up: apparently none The follow-up time for newborns is unclear.	No	
		Unclear	
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?	Yes	<b>X</b>
	It is not necessary	No	
		Unclear	
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention?	Yes	
	No, they include all participants with syphilis	No	<b>X</b>
		Unclear	
1.16	Are confidence intervals for the absolute effect of the intervention presented	Yes	<b>X</b>
	CI 95 it is presented	No	
		Unclear	
<b>Section 2 – Overall Evaluation of the Study</b>			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	
		No	
		Unclear	<b>X</b>
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	<b>X</b>
		No	
		Unclear	

**Risk of Bias Assessment**

Selection bias: Low

Performance and detection bias: High(1.8, 1.12)

Attrition bias : Low

Confounding: High

Other problems : CI 95% is not calculated

Overall risk. Very high

Checklist for series of subjects exposed to intervention studies			
Reference: Watson-Jones D, Syphilis in pregnancy in Tanzania. II. The effectiveness of antenatal syphilis screening and single-dose benzathine penicillin treatment for the prevention of adverse pregnancy outcomes. J Infect Dis. 2002 ;186(7):948-57. doi: 10.1086/342951			
Checklist verified by:			
<b>Section 1 Internal validity</b>			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
1.1	Does the study define an appropriate, clear, and focused research question? We examined whether single-dose benzathine penicillin treatment is adequate to prevent adverse pregnancy outcomes in the Mwanza region of north-western Tanzania, where syphilis screening is now being implemented using the single-dose benzathine penicillin regimen and where most pregnant women attend ANC's after 20 weeks of gestation	Yes	<b>X</b>
		No	
		Unclear	
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention? From September 1997 through November 1999, all eligible RPR-positive women screened and treated with single-dose benzathine penicillin, followed by the next 2 eligible RPR-negative women attending the ANC, were recruited. Inclusion criteria included informed consent, residence in Mwanza Municipality for at least 1 month, and ultrasound-proven viable pregnancy.	Yes	<b>X</b>
		No	
		Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention? Women with a multiple gestation (i.e., 11 foetus), penicillin allergy, medical complications of pregnancy (hypertension, diabetes, or prior vaginal bleeding), or congenital foetal abnormal	Yes	<b>X</b>
		No	
		Unclear	
1.4	What proportion of eligible subjects participated in the study? 1361 Women with RPR (+) were candidates. 805 RPR (+) were not included were not eligible, predominantly because they were not residents of Mwanza or were planning to leave prior to delivery (55%) Then 556 RPR (+) were included (45%).154 were excluded Were analysed 402 = 153 (high titre) + 249 (low titre) (30%)	Yes	<b>X</b>
		No	
		Unclear	
1.5	Were exposed subjects included in strict sequential order? There is no information	Yes	
		No	<b>X</b>
		Unclear	
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention? Table 1	Yes	<b>X</b>
		No	
		Unclear	

1.7	Is the intervention applied to the subjects clearly defined?	Yes	X
	Women who were seronegative for syphilis at the initial ANC screening who were later found to be RPR positive after retesting of serum samples at the reference laboratory were treated for syphilis with benzathine penicillin G (2.4 MUim).	No	
		Unclear	
1.8	Is the intervention described in sufficient detail to allow replication (definition and components)?	Yes	
	There is no informed	No	
		Unclear	X
1.9	Were any co-interventions applied differentially to the exposed subjects?	Yes	X
	Recommended treatments at that time included metronidazole for the treatment of T. vaginalis and bacterial vaginosis, cotrimoxazole for the treatment of gonorrhoea and genital ulceration, erythromycin for the treatment of Chlamydia trachomatis, and clotrimazole pessaries for the treatment of vaginal candidiasis if those were tested as (+)	No	
		Unclear	
1,10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?	Yes	
	Open label study	No	X
		Unclear	
1.11	Are prognostic variables adequately defined and measured?	Yes	
	No time between Treatment and delivery nor reinfection	No	
		Unclear	X
1.12	Are outcome variables adequately defined and measured?	Yes	X
	The infant was examined and weighed. Stillbirth was defined as a foetal death at 122 weeks of gestation, and intrauterine foetal death (IUFD) was defined as foetal death at 22 weeks of gestation. LBW was defined as birth weight of !2500 grams, preterm birth was defined as delivery at !37 weeks of gestation, and intra uterine growth retardation (IUGR) was defined as LBW after 37 weeks of gestation [21–23]. Gestational age at birth was estimated by 2 methods, ultrasound/LMP dating or a standard Dubovitz ex amination [24] performed on live-born infants within 5 days of birth	No	
		Unclear	
1.13	What proportion of subjects were lost to follow-up?	Yes	X
	42/462 = 12% (Women lost to follow-up before delivery were younger than those followed up (mean age, 22.7 vs. 23.8 years; Pp.01 (41.3% vs. 28.3%) and were more likely to be primigravidae Pp.03 ) and single (24.7% vs. 14.2%; p < 0.05)	No	
		Unclear	
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?	Yes	
	Authors did not perform causal inference analysis	No	X
		Unclear	

1.15	Is there an estimation of the required sample size to determine the benefit of the intervention?	Yes	
		No	<b>X</b>
		Unclear	
1.16	Are confidence intervals for the absolute effect of the intervention presented CI are not provided	Yes	
		No	<b>X</b>
		Unclear	
<b>Section 2 – Overall Evaluation of the Study</b>			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	<b>X</b>
		No	
		Unclear	
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	<b>X</b>
		No	
		Unclear	

**Assessment**

Selection Bias: High (1.4)

Performance and detection bias : Low

Attrition Bias: High (1.13)

Confusion Bias: High (1.14)

Other problems Yes

Overall Risk: Very high

<b>Checklist for series of subjects exposed to intervention studies</b>			
Reference: Zhang XH, Xu J, Chen DQ, Guo LF, Qiu LQ. Effectiveness of treatment to improve pregnancy outcomes among women with syphilis in Zhejiang Province, China. Sex Transm Infect. 2016 Nov;92(7):537-541. doi: 10.1136/sextrans-2015-052363.			
Checklist verified by:			
<b>Section 1 Internal validity</b>			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
1.1	Does the study define an appropriate, clear, and focused research question? The purpose of this study was to determine the prevalence of maternal syphilis, treatment effectiveness and syphilis-associated pregnancy outcomes.	Yes	<b>X</b>
		No	
		Unclear	
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention? All pregnant women with syphilis who delivered between 1 January 2013 and 31 December 2014 were enrolled. We recorded women's pregnancy outcomes, including live births and perinatal deaths (still birth at $\geq 28$ weeks and 0–7 days for neonatal deaths)	Yes	<b>X</b>
		No	
		Unclear	
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention? Women who terminated their pregnancies voluntarily, who had multiple pregnancies or suffered early miscarriage (<28 weeks) and without clear treatment information were excluded from the Study	Yes	<b>X</b>
		No	
		Unclear	

1.4	What proportion of eligible subjects participated in the study?	Yes	
	There is no information on the candidate population and exclusions	No	
		Unclear	X
1.5	Were exposed subjects included in strict sequential order?	Yes	X
	Apparently, all mothers and newborns who met the inclusion criteria (3767)	No	
		Unclear	
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention? Table 1	Yes	X
		No	
		Unclear	
1.7	Is the intervention applied to the subjects clearly defined?	Yes	X
	If women chose to continue pregnancies, they were provided free treatment with two courses of penicillin injections in the first trimester and the third trimester, respectively, such as 2.4 million units of benzathine penicillin by weekly injection for 3 weeks or 0.8 million units of procaine penicillin by daily injection for 15 days per treatment course. Erythromycin or ceftriaxone was provided to those women who were allergic to penicillin,	No	
		Unclear	
1.8	Is the intervention described in sufficient detail to allow replication (definition and components)?	Yes	X
	We categorized women according to treatment regimens: whether treated or not, whether treated adequately or inadequately and the trimester of treatment initiation. The treated group included all women with syphilis who received at least one dose of penicillin/erythromycin/ceftriaxone during pregnancy. Women who did not receive treatment during the entire pregnancy period were considered the untreated group. Women with two courses of penicillin injections and at least 2 weeks off between the two courses were categorised in the adequate treatment group. Others who received non-penicillin treatment or were treated with fewer than two courses were classified in the inadequate treatment group.	No	
		Unclear	
1.9	Were any co-interventions applied differentially to the exposed subjects?	Yes	
	There is no information	No	
		Unclear	X
1,10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?	Yes	
	Open label study. Hard outcomes	No	X
		Unclear	
1.11	Are prognostic variables adequately defined and measured?	Yes	
	No Gestational age No time between Treatment and delivery	No	
		Unclear	X

1.12	Are outcome variables adequately defined and measured?	Yes	X
	CS was diagnosed in infants who met any one of the following criteria: (1) infants with a titre in the TRUST or RPR tests four or more times higher than his or her mother's values during labour (however, the absence of a fourfold increase could not exclude CS); (2) infants with abnormal physical examinations consistent with syphilis infection; (3) infants with positive TPPA/TPHA tests lasting until 18 months after birth or (4) infants with positive IgM or T. pal lidum.	No	
		Unclear	
1.13	What proportion of subjects were lost to follow-up? There were no losses	Yes	X
		No	
		Unclear	
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?	Yes	
		No	X
	There was no use of inference causal analysis	Unclear	
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention? It is not relevant	Yes	
		No	
		Unclear	X
1.16	Are confidence intervals for the absolute effect of the intervention presented CI 95% were calculated	Yes	X
		No	
		Unclear	
<b>Section 2 – Overall Evaluation of the Study</b>			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	X
		No	
		Unclear	
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	X
		No	
		Unclear	

## Assessment

Selection Bias: Unclear

Performance and detection bias High (1.8)

Attrition Bias: Low

Confusion Bias: High

Other problems: CI 95% was not calculated

Overall Risk: Very high

Checklist for series of subjects exposed to intervention studies			
Reference: Zhu L, Qin M, Du L, Xie RH, Wong T, Wen SW. Maternal and congenital syphilis in Shanghai, China, 2002 to 2006. Int J Infect Dis. 2010 ;14 Suppl 3:e45-8. doi: 10.1016/j.ijid.2009.09.009			
Checklist verified by:			
<b>Section 1 Internal validity</b>			
In this case series (exposed) study: (Please include the relevant excerpt from the evaluated study.)		In this study the criterion is: (Mark the selected answer with an X)	
1.1	Does the study define an appropriate, clear, and focused research question?	Yes	<b>X</b>
	To assess the trends and determinants of maternal and congenital syphilis in Shanghai, China.	No	
		Unclear	
1.2	Is there a clear description of the inclusion criteria for subjects exposed to the intervention?	Yes	
	All pregnant women who had their prenatal care services provided by a hospital or a clinic in Shanghai and delivered their babies in Shanghai between January 1, 2002 and December 31, 2006	No	
		Unclear	<b>X</b>
1.3	Is there a clear description of the exclusion criteria for subjects exposed to the intervention?	Yes	
	There is no information	No	
		Unclear	<b>X</b>
1.4	What proportion of eligible subjects participated in the study?	Yes	<b>X</b>
	100%	No	
		Unclear	
1.5	Were exposed subjects included in strict sequential order?	Yes	<b>X</b>
	All women with GS were included	No	
		Unclear	
1.6	Was a baseline measurement of the characteristics of the exposed subjects conducted to establish their condition prior to the intervention?	Yes	
	No description of Characteristics of women with GS	No	
		Unclear	<b>X</b>
1.7	Is the intervention applied to the subjects clearly defined?	Yes	<b>X</b>
	For primary, secondary, and early latent syphilis, we used Benzathine penicillin G (4.8 million units) Intramuscularly in two doses (9.6 million units in total) weekly. For late latent syphilis, we used benzathine penicillin G (2.4 million units) intramuscularly in three doses (7.2 million units in total) Weekly.	No	
		Unclear	

1.8	Is the intervention described in sufficient detail to allow replication (definition and components)? Maternal syphilis cases who received a whole course of penicillin treatment was considered completely treated, and those who did not complete a whole course of Treatment were considered incompletely treated. Women with less or more of 28 weeks were compared  Do not take into account the effect of incomplete treatment on the incidence of SC	Yes	
		No	<b>X</b>
		Unclear	
1.9	Were any co-interventions applied differentially to the exposed subjects?  There is no information	Yes	
		No	
		Unclear	<b>X</b>
1.10	Were measures taken to prevent prior knowledge of exposure from influencing outcome assessment?  Open label study. There is not a clear definitions of GS	Yes	
		No	<b>X</b>
		Unclear	
1.11	Are prognostic variables adequately defined and measured?  Gestational age No time between Treatment and delivery	Yes	<b>X</b>
		No	
		Unclear	
1.12	Are outcome variables adequately defined and measured?  CS: Congenital syphilis is syphilis in an infant whose mother has transmitted the infection vertically to the foetus. Relevant demographic and clinical data for maternal syphilis and congenital syphilis cases were collected using structured forms	Yes	
		No	<b>X</b>
		Unclear	
1.13	What proportion of subjects were lost to follow-up?  Apparently, there were no losses	Yes	
		No	
		Unclear	<b>X</b>
1.14	Are the main confounding factors identified and accounted for in the analysis (e.g., causal inference methods)?  Authors did no uses causal inference analysis	Yes	
		No	<b>X</b>
		Unclear	
1.15	Is there an estimation of the required sample size to determine the benefit of the intervention?  All base population was included	Yes	<b>X</b>
		No	
		Unclear	
1.16	Are confidence intervals for the absolute effect of the intervention presented  Not provided	Yes	
		No	<b>X</b>
		Unclear	

Section 2 – Overall Evaluation of the Study			
2,1	Taking into account clinical considerations and your evaluation of the methodology used, do you consider that the observed effect is probably due to the investigated exposure?	Yes	
		No	
		Unclear	X
2,2	Are the results of the study directly applicable to the target population of the systematic review?	Yes	X
		No	
		Unclear	

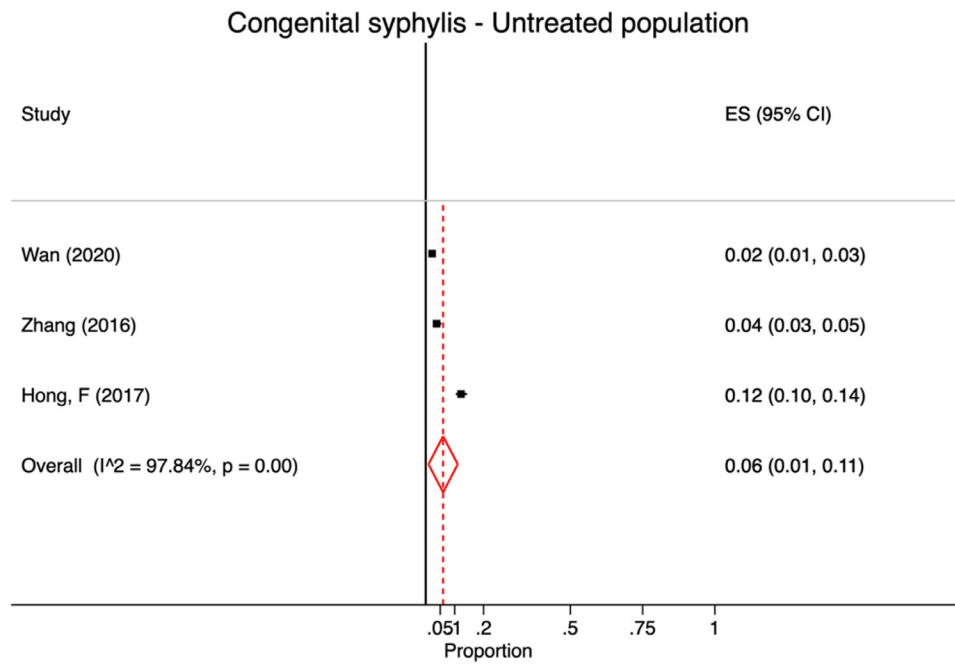
Risk of Bias Assessment

Selection Bias: Unclear  
 Performance and detection bias: High  
 Attrition Bias: low  
 Confounding: High  
 Overall Risk: Very High

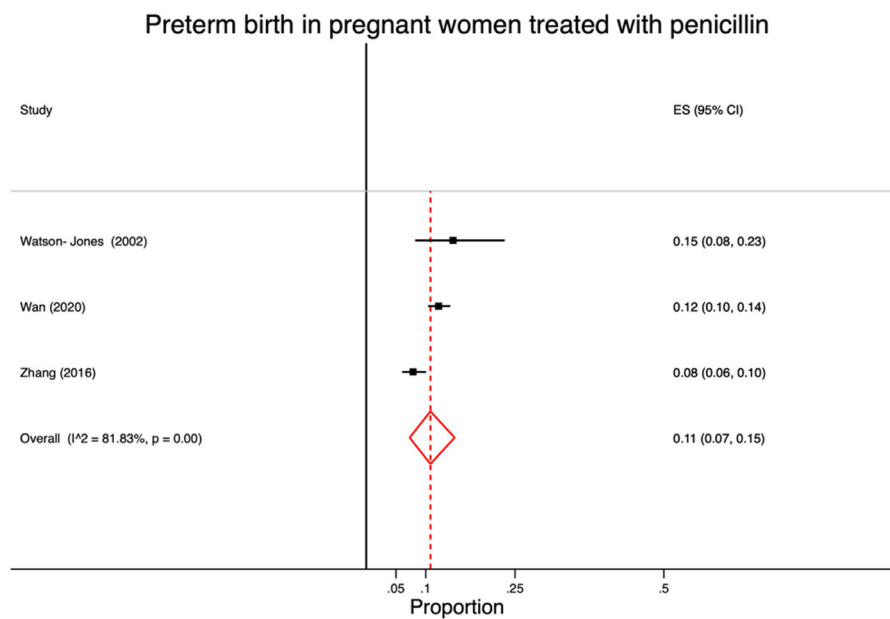
**APPENDIX 6. RISK OF BIAS OF CASES SERIES (JBI TOOL)**

Appendix 5. Risk of bias of cases series (JBI tool)			
In thi	Study		
	Camacho et al. <sup>36</sup> 2021	Sheffield J, et al. <sup>37</sup> 2002	Yanase et al. <sup>38</sup> 2025
Were there clear criteria for inclusion in the case series?	Yes	Unclear	Yes
Was the condition measured in a standard, reliable way for all participants included in the case series?	Yes	Unclear	Yes
Were valid methods used for identification of the condition for all participants included in the case series?	Yes	Yes	Yes
Did the case series have consecutive inclusion of participants?	Unclear	Yes	Yes
Did the case series have complete inclusion of participants?	Unclear	Yes	Yes
Was there clear reporting of the demographics of the participants in the study?	Yes	Yes	Yes
Was there clear reporting of clinical information of the participants?	Yes	Yes	Yes
Were the outcomes or follow up results of cases clearly reported?	Yes	No	Yes
Was there clear reporting of the presenting site(s)/ clinic(s) demographic information?	Yes	Yes	No
Was statistical analysis appropriate?	Not applicable	Not applicable	Not applicable
Overall appraisal:	Include	Include	Include
Total (out of 10)	7	6	9

## APPENDIX 7. ADDITIONAL FIGURES



**Figure S1:** Heterogeneity assessment: incidence of congenital syphilis in untreated women with gestational syphilis



**Figure S2:** Heterogeneity assessment: incidence of preterm birth in women with gestational syphilis treated with penicillin