REVIEW ARTICLE

Association of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with female infertility: a systematic review and meta-analysis

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ABSTRACT

BACKGROUND
Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (NG) are commonly diagnosed sexually transmitted infections that have been associated with serious reproductive health outcomes for women. The association of CT and NG infection with female fertility is not completely established yet. This review aimed to determine the association of CT and NG with female infertility.

METHODS
This systematic review and meta-analysis was conducted according to the PRISMA statement. We searched a range of electronic databases, including PubMed, Web of Science, Embase, and Scopus, from Sept 25, 2017 until February 1, 2021. From the 851 studies screened, 552 that failed to meet our eligibility criteria were excluded. Subsequently, we removed 290 studies for not having a possible correlation of CT and NG infections with female infertility. Nine studies comprising 1827 infertile patients met our inclusion criteria. Two investigators independently extracted a range of data. All analyses were performed using STATA (version 13.1, Stata Corp, College Station, TX, USA).

RESULTS
CT infection potentiates female infertility, as 76.47% of the included studies found a positive correlation between them. However, due to the limited number of reported data, we were not able to compare NG infection prevalence in fertile and non-fertile patients. Overall prevalences of CT and NG infections among infertile patients were 12 % and 3%, respectively, while CT infection prevalence among the fertile group was 7%.

CONCLUSION
The prevalences of CT and NG infections were high in infertile women. Screening and treatment of *C. trachomatis* and gonococcal infections during infertility treatment might increase the pregnancy rate.

Keywords: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, female infertility, human reproduction

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INTRODUCTION

Sexually transmitted infections (STIs) are among the most common acute conditions around the world. [1] It was estimated in 2016 by the World Health Organization (WHO) that there were approximately 370 million new cases of the three curable STIs worldwide.[1] *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are commonly diagnosed sexually transmitted infections (STI) that have been associated with serious reproductive health outcomes for women, and may cause obstetric or perinatal complications,[2-4] including preterm deliveries, preterm rupture of the membranes, low birth weight, or still-birth. Tubal factor infertility (TFI) and ectopic pregnancy have been associated with a history of both STIs in numerous studies. [5,6] Despite these burdensome sequelae, STI control has long languished on health policy agendas. The 2030 Agenda on Sustainable Development [7] aimed to remedy this situation and led to WHO's Global Health Sector Strategy on STIs.[8] The strategy proposes an integrated approach for STI prevention and control that addresses core Sustainable Development Goals, mainly through securing universal access to sexual and reproductive health-care services and rights.[7,8] The first strategic direction of this STI strategy is "the need to understand the sexually transmitted infection epidemic and response as a basis for advocacy, political commitment, national planning, resource mobilization and allocation, implementation, and program improvement".[8] Longitudinal studies examining gonorrhea's adverse health outcomes (a curable infection) are difficult/unethical to conduct. A recent study attempted to overcome this challenge through linking national testing databases to hospital records, but identified too few cases to reach conclusive evidence about gonorrhea's role in infertility.[9] In the absence of direct evidence, our review aimed to provide indirect evidence for a link between gonorrhea and infertility but strictly did not aim to nor can it establish causality. The underlying hypothesis is that current infection is of unknown duration and persistence and therefore does not allow the establishment of a causal link with infertility, but is often predictive of past exposure.[10-12] This assertion is supported by several lines of evidence. It is established through tens of studies of different designs that gonorrhea as well as chlamydia, being curable infections, carry a high risk of reinfection because of re-exposure to the same sexual partner or to other high-risk partners.[11,13-15] As such, it can be assumed that a current gonorrhea infection is strongly indicative of previous gonorrhea infection;[11,16,17] indeed studies have shown that the strongest predictor of current gonorrhea infection is a history of gonorrhea infection.[10,18] For example, in the UK, a history of gonorrhea infection was found to be the strongest predictor of current gonorrhea infection even after controlling for other demographic and behavioral factors (adjusted OR 4.36, 95% CI 1.78 to 10.71).[10] Globally, it has been estimated that the prevalence of *N. gonorrhoeae* is 0.8% and 0.6% among women and men, respectively.[1] For the Eastern Mediterranean Region, the WHO has reported prevalence rates of chlamydia, gonorrhea, and trichomoniasis among women of 3.8%, 0.7%, and 4.7%, respectively.[1] It is estimated that up to 40% of women with untreated *C. trachomatis* infection will develop pelvic inflammatory disease (PID) which is defined as any combination of endometritis, salpingitis, tubo-ovarian abscess, or pelvic peritonitis.[19] Of those with PID from chlamydial or gonococcal sources, 20% will become infertile, 18% will experience debilitating chronic pelvic pain and 9% will have a life-threatening ectopic pregnancy.[19-22]

A case-control study did not show an association of active infection due to *C. trachomatis* and *N. gonorrhoeae* with secondary infertility in females.[20] A previous systematic review corroborated the hypothesis that *C. trachomatis* infection potentiates female infertility, as 76.47% of their included studies

found a positive correlation between them.[21] Even though this issue is widely recognized amidst the medical community as a secondary effect of female *C. trachomatis* and gonococcal infection, the level of evidence corroborating the association is relatively weak. An updated systematic review and meta-analysis of these reported data may help to better evaluate the etiologic role of *C. trachomatis* and *N. gonorrhoeae* in female infertility. The objective of this review was to verify the scientific investigations related to the association of *C. trachomatis* and gonococcal infection with female infertility.

METHODS

This review was conducted in accordance with the PRISMA statement.[23]

Search strategy

We searched a range of electronic databases, including PubMed, Web of Science, Embase, and Scopus, from Sept 25, 2017 to February 2021. The search terms used were as follows: [(infertility OR sterility OR subfertility) AND (*genital OR vagin*) AND (Chlamydia trachomatis OR Neisseria gonorrhoeae) AND (microbiota OR microbiota OR vaginosis OR lactobacilli)]. The citation lists of any identified publications were also searched manually to identify any additional references. Our searches did not involve any restrictions related to language or country of origin.

Inclusion and exclusion criteria

Titles and abstracts were first reviewed by two of the authors and only relevant publications were selected for full review. Studies in this meta-analysis were required to meet the following inclusion criteria: (1) observational studies that addressed infertile women and fertile controls; (2) the vaginal microbiota status was characterized by 16S rRNA gene amplicon sequencing, Nugent score, Amsel's criteria, or Spiegel's criteria; (3) human studies; and (4)

original studies that provided clear data relating to vaginal microbiota status. Articles were excluded if they were: (1) comments, reviews or conference abstracts; (2) repetitive studies; (3) animal studies; (4) devoid of a control group; (5) linked to a control group that was not made up of fertile women; (6) the status of the vaginal microbiota was based on diagnosis of bacterial vaginosis without any specific methodology; (7) not written in English or Chinese; (8) studies related to clinical intervention.

Data extraction

The two above-mentioned reviewers independently extracted a range of data, including date of publication, authors, study design, study population, sample size, methods used for microbiota characterization/diagnosis, and the specific type of infertility. Any disagreements were resolved through discussion with a third reviewer.

Quality assessment

The two reviewing authors evaluated the quality of the studies included in our analyses based on the Newcastle–Ottawa Scale (NOS), which considers three critical aspects: selection, comparability, and exposure. The two investigators scored the studies independently and any discrepancies between reviewers were resolved by reaching a consensus or by a fourth reviewer.

Statistical analysis

Associations between the vaginal microbiota and infertility were estimated by pooled odds ratios (ORs) and 95% confidence intervals (CIs) using the Mantel–Haenszel method. Heterogeneity between studies was tested using Cochran's Q two-sided homogeneity test; the I2 statistic was also used as a critical factor to determine which model should be used to pool the effect size (if I2 <50%, then a fixed model was used; otherwise, a random model was used). Publication bias was then evaluated by Begg's funnel plots and Egger's regression test, which measures the

degree of funnel plot asymmetry. Subgroup analyses were performed based on the language used to write the original papers, the different methods used to diagnose vaginal microbiota, different types of infertility, and fertile control groups, to reduce heterogeneity. Several sensitivity analyses were performed to evaluate the robustness of our results by excluding some articles that were considered to be of low quality. All analyses were performed using STATA (version 13.1, StataCorp, College Station, TX) and the 'metan' command was used to estimate the ORs. A two-sided $p<0.05$ was considered to be statistically significant.

RESULTS

Study selection and characteristics

In total, we screened 1155 records for eligibility from which 104 were duplicates. Out of the remaining 1051 papers , 200 articles excluded based on the irrelevant abstracts, 17 articles were review, 51 have focused on other populations, 7 were letter to editors , 49 did not provide an available full text, and 428 articles did not provide specific data, leaving 229 full texts to be assessed fully. After reviewing these 229 papers, only 9 studies met our inclusion criteria for meta- analysis. and the rest were excluded because of none sufficient data or missing the outcome of interest (Figure 1).

Collectively, these 9 articles reported 1827 cases. Because infertility status and vaginal microbiota data were collected at the same time, it was hard to infer a causal correlation between these factors. Hence, we considered these articles as cross-sectional rather than case–control studies, despite their original definition. Most of the studies (n=8) focused on women who were diagnosed with infertility and seeking clinical treatment; in these studies, the specific type of infertility was ignored. We also found that the control (fertile) groups were quite diverse. For example, some papers used women with a history of pregnancy, antenatal women, and even healthy women without a detailed history of pregnancy. Further details of the included studies are shown in Table 1.
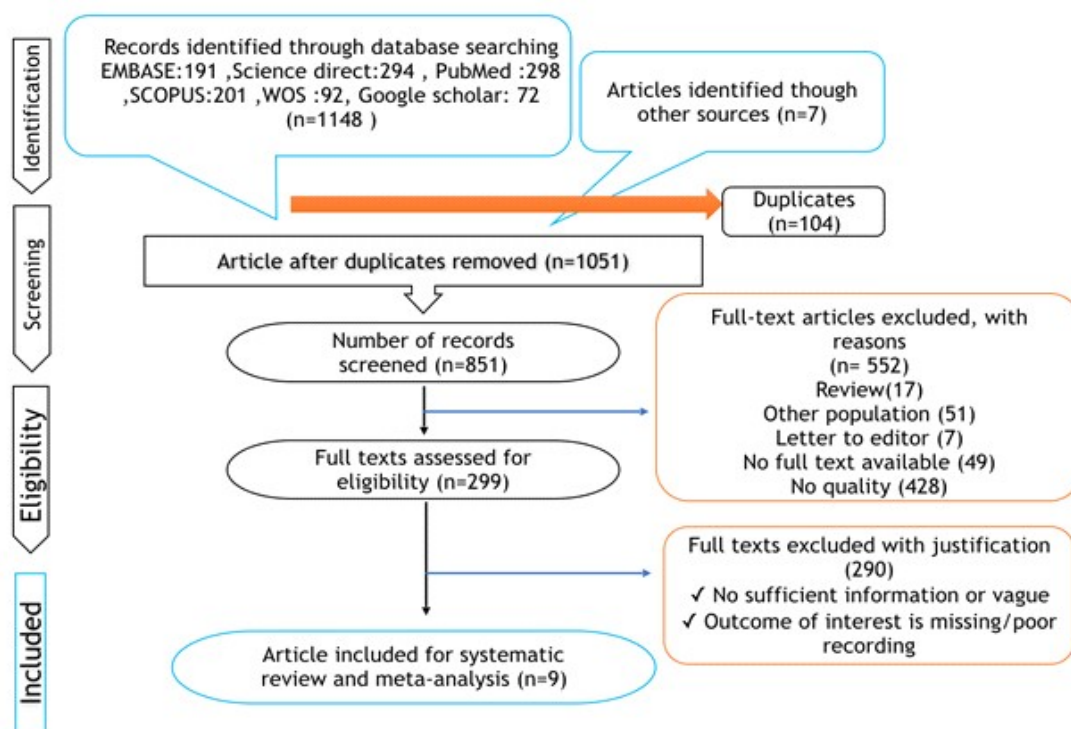

Figure 1. The PRISMA flow diagram: study selection process

Table 1. Study characteristics

Author	Year	Country	Patients	F	M	Age	Chlamydia		Gonorrhea		Design	Study period	Mean infertility duration	Type of Infertility	
							Infertile	Fertile	Fertile	Infertile				Primary	Secondary
Nwankwo [48]	2014	Nigeria	125	69	56	14-55	42.7%	N/A	N/A	N/A	Pros	June 2012-Dec 2012	N/A	N/A	N/A
Akinnibosun [49]	2018	Nigeria	50	50	0	24-51	N/A	N/A	N/A	N/A	Pros	Jan2015-March2015	N/A	32%	68%
Adewunmi [50]	2018	Ikeja	147	147	0	36.5±5.6	48%	25.9%	N/A	N/A	Pros	N/A	N/A	8.3%	91.7%
Kamel [51]	2013	Saudi Arabia	640	640	0	18-40 (26.4 ± 4.8)	15%	N/A	N/A	N/A	N/A	2011-2012	3.28 ± 1.73 years	N/A	N/A
Sameni [52]	2017	Iran	65	65	0	20-40 (24.3)	13.8%	N/A	6.2%	N/A	Pros	2016-2017	N/A	N/A	N/A
Marashi [53]	2013	Iran	150	150	0		15.3%	3.5%	N/A	N/A	Pros	N/A	N/A	N/A	N/A
Cheong [54]	2019	Malaysia	70	N/A	N/A	20-44 (31.4)	40/70	N/A	N/A	N/A	Pros	2010-2014	N/A	N/A	N/A
M. Elkayal [55]	2015	Egypt	100	100	0	20-45	2.67%	0	2%	1.3%	Pros	N/A	N/A	N/A	N/A
Reekie [9]	2019	Australia	473	473	0	N/A	8%	N/A	3.3%	N/A	Retro	2001-2013	N/A	N/A	N/A

Abbreviations: F: female; M: male; N/A: not applicable; Pros: prospective; Retro: retrospective
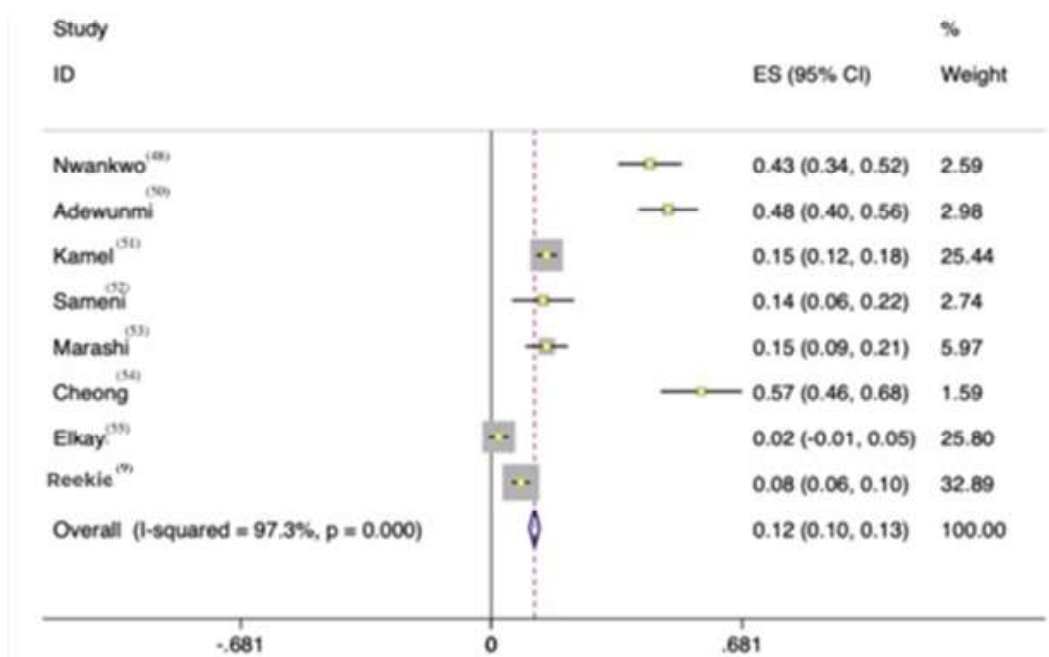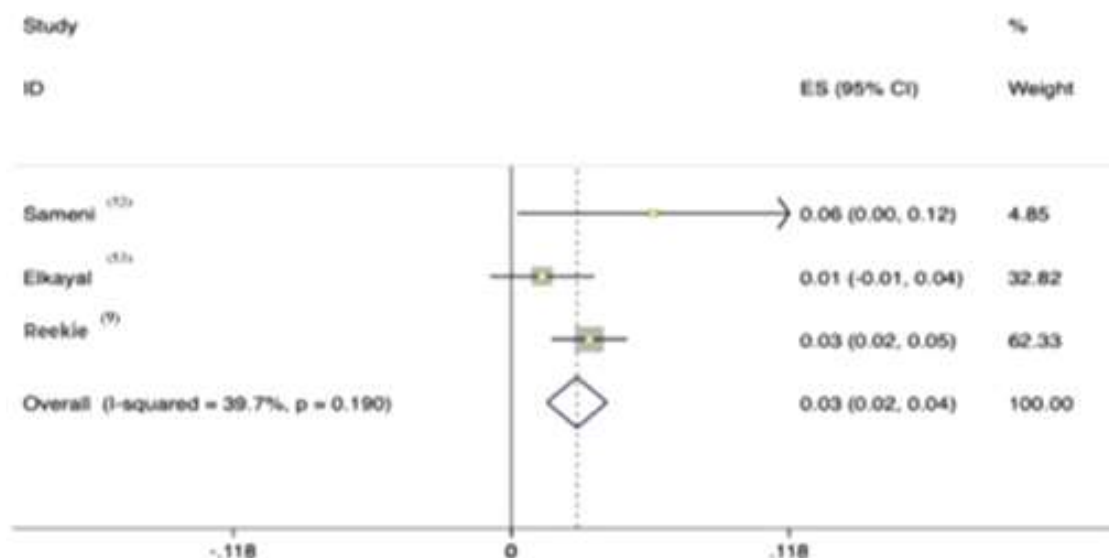
Abbreviation: ES = effect size

Figure 2. The overall prevalence of *Chlamydia trachomatis* among infertile patients

Overall prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among infertile patients

The overall prevalence of *C. trachomatis* and gonococcal infections among infertile patients was 12% and 3%, respectively, while the prevalence of *C. trachomatis* infection among the fertile group was 7% (Figures 2 and 3).
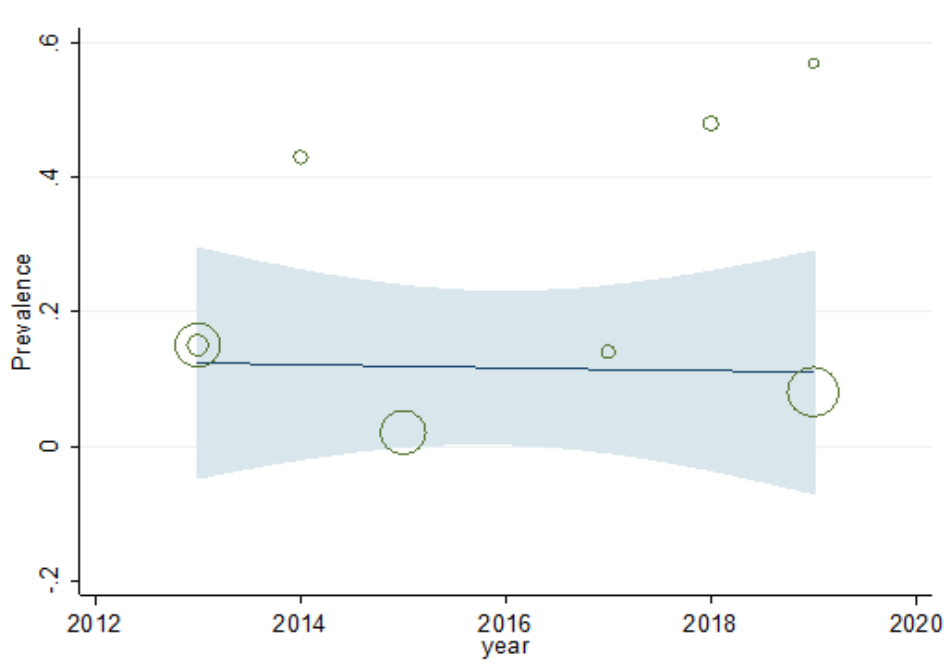
Meta-regression finding based on the publication year and prevalence of *Chlamydia trachomatis* among infertile women

The studies' meta-regression was according to the association between the prevalence of *Chlamydia trachomatis* among infertile women and the publication year of the study. It showed

Abbreviation: ES = effect size

Figure 3. The overall prevalence of *Neisseria gonorrhoeae* among infertile patients

Figure 4. Meta-regression finding based on the publication year and prevalence of *Chlamydia trachomatis* among infertile women

the overall rate of infection was lower in newer studies than in older ones (Figure. 4). However, there was no statistically significant linear trend in univariate meta-regression to explain effect size variation by publication year of study with coefficient (Figure 4).

Meta-regression finding based on the mean age of patients and prevalence of *Chlamydia trachomatis* among infertile women

The studies' meta-regression was according to the association between mean age of patients and prevalence of *Chlamydia trachomatis* among infertile women. It showed that the overall rate of infection was lower at younger than older ages (Figure 5). However, there was no statistically significant linear trend in univariate meta-regression to explain effect size variation by mean age of patients with coefficient (Figure 5).

Publication bias

In Figure 6, the funnel plot of publication bias is shown to be symmetrical. The size of the circles shows the weight of the studies (larger circles show more samples and smaller circles show fewer samples).

DISCUSSION

The WHO estimates that the main curable STIs (gonorrhea and chlamydial infection) are commonest in the 15-44-year age groups.[24] They are the most common causes of cervicitis and urethritis, and their sequelae (pelvic inflammatory disease, chronic pelvic pain, tubal factor infertility, and reactive arthritis).[25] Chlamydial infections are primarily an issue of women's health care since the manifestations and consequences are more damaging to the reproductive health in women than in men.[26] Most urogenital *C. trachomatis* and gonococcal infections are initially asymptomatic but may subsequently cause considerable long-term morbidity. Consequently, accurate diagnosis of both infections requires the use of specific laboratory techniques. The important progress in laboratory diagnosis of chlamydial infection includes the development of non-viability-dependent tests.[27] We provided, to our
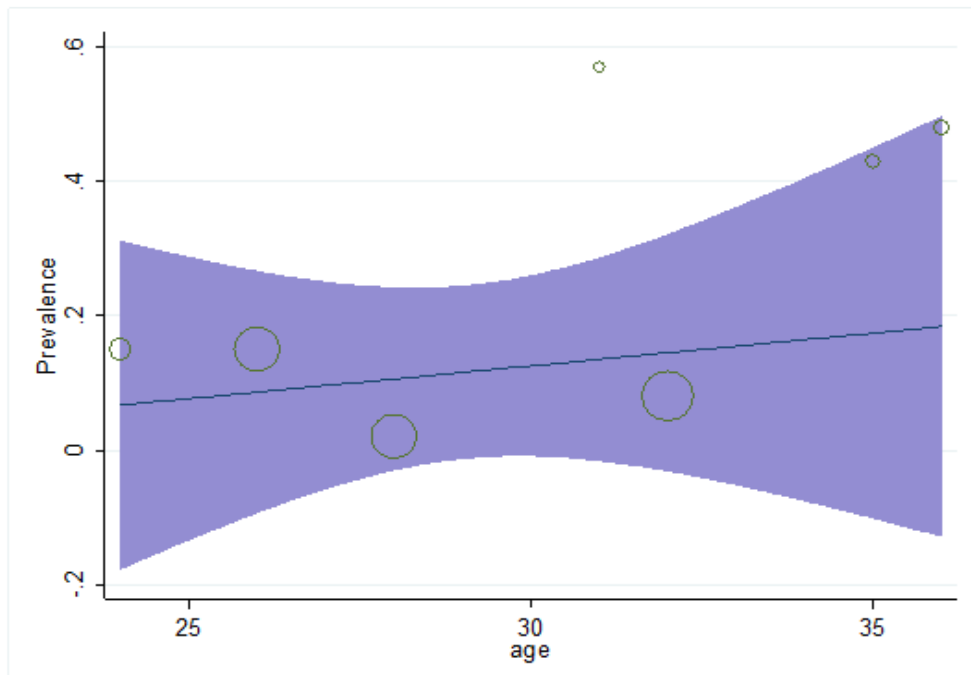
Figure 5. Meta-regression finding based on the mean age of patients and prevalence of *Chlamydia trachomatis* among infertile women

knowledge, the first systematic review of *C. trachomatis* and gonorrhea infection in infertile populations. The gonorrhea infection prevalence was several folds higher than that in the general population; the global estimate in infertile populations was 2.2%, compared with only 0.8% in the general population (per WHO 2016 estimates),[28] but in our study this rate was 12%. These findings should be seen against the expectation that infertile populations should be prone to a lower prevalence than the general population; there is higher frequency of STI testing among them, and therefore earlier detection and higher treatment coverage relative
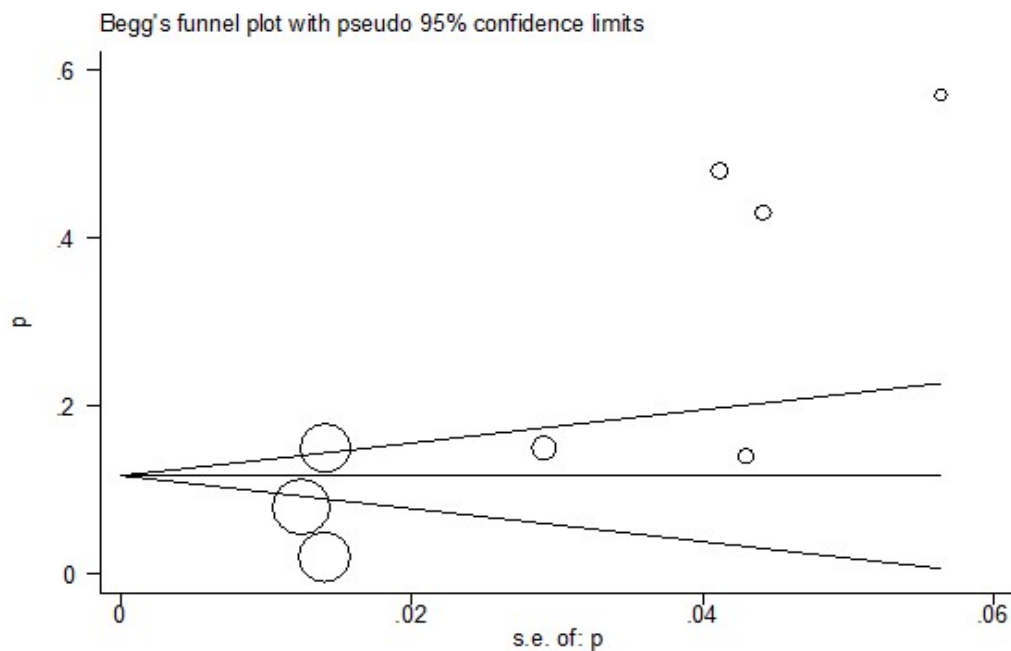
Figure 6. Begg's funnel plot

to the general population. Infertile populations may also undergo prophylactic antibiotic administration, not necessarily with testing, prior to procedures such as in vitro fertilization/embryo transfer.[29,30] It is relatively clear that some sexually transmitted infections (STIs) can cause infertility. *Chlamydia. trachomatis* may synthesize a large amount of heat shock protein (hsp60) and thus induce a pro-inflammatory immune response in the human fallopian tube epithelium, resulting in scarring and tubal occlusion.[31] *N. gonorrhoeae* may attack the epithelial cells of the fallopian tubes, thus exerting an impact on ovum transportation and fertilization.[32] Our study revealed a prevalence of 12% for *C. trachomatis* among infertile women. These findings attest to the potential role of *C. trachomatis* and gonorrhea in infertility. Since early detection and treatment of *C. trachomatis* and gonococcal infections have been challenged by the asymptomatic nature of the infections [33,34] and growing antimicrobial resistance,[35-40] these findings support the global public health value of developing STI vaccines [41,42] as a fundamental solution to the implications of *C. trachomatis* and gonorrhea.[43]

Our results also support the timeliness of a comprehensive prevention approach promoting sexual health to control *N. gonorrhoeae* and *C. trachomatis*, mitigate antimicrobial resistance and achieve WHO global health sector strategy targets. [35] Such an approach would focus on the simultaneous implementation of biomedical (rolling-out testing and vaccination), behavioral (promoting healthier sexual lives) and structural prevention interventions (improving access to testing, treatment and care services). Indeed, successful and sustainable implementation of biomedical interventions cannot be achieved without adequate levels of public awareness, access to/uptake of services, and adherence/ retention in prevention and treatment cascades. *Chlamydia trachomatis* current infection prevalence was 3% in the Middle East and North Africa in 2020, similar to WHO prevalence estimates for this region of about 3% in 2012 and

about 3.5% in 2016.[44] The prevalence was also in line with WHO estimates for the Western Pacific region (about 4%) and the European region (about 3%),[44] where broad *C. trachomatis* control programs, including opportunistic testing, are standard in some high-income countries,[45-47] but higher than that for the South-east Asian region (about 1.5%) and lower than that for the African region (about 5%) and the region of the Americas (about 5.5%).[44] This high prevalence suggests substantial infection and disease burden that needs to be tackled through sexual health and STI -specific programs, for both women and men. Our study has important but unavoidable limitations. Data quantity and quality varied across regions and sometimes limited our ability to produce representative summary estimates. It was not possible to conduct full multivariable meta-regression to adjust for potential confounders, with the large number of predictors relative to that of studies. Prevalence estimates by infertility diagnosis may have been affected by unavoidable overlap across categories; samples with mixed infertility often included tubal factor infertility (TFI), and those with non-TFI may have included other infertility diagnoses. An analysis by age could not be performed, given the low number of studies reporting patients' age. Quantity and quality of available data varied by country and population, particularly for populations at high risk where most data came from only a few countries. Prevalence levels might not have been strictly representative and might have been affected by publication bias, as suggested by the small -study effect observed. The wide array of diagnostics used for ascertainment might have also introduced detection bias. Factors that might have contributed to differences in *C. trachomatis* positivity rates across studies include sampling variation and potential selection bias, spatial or temporal variability in prevalence, and possibly unreported underlying comorbidities. This study did not assess other STIs that might have also contributed to infertility, pregnancy- related morbidity, and other health conditions in women with *C. trachomatis* or gonorrhea infection. Such

potential biases might have contributed to some of the unexplained heterogeneity observed in the prevalence levels. Given the potential limitations in the representativeness of the prevalence measures as well as heterogeneity across studies, the calculated pooled prevalence should be interpreted as a pooled average, rather than strictly an estimate of the mean prevalence in the considered population or subpopulation.

CONCLUSION

The prevalence of *C. trachomatis* and gonococcal infections were respectively high in infertile women. These infections are strongly implicated in female infertility and are probably an underestimated cause of unexplained infertility. Screening and treatment of *C. trachomatis* and gonococcal infections during the course of infertility treatment might increase pregnancy rate. Randomized studies including larger numbers of participants are needed, however, to reach more validated conclusions. Moreover, research is strongly recommended on the mechanisms by which *C. trachomatis* and gonococcal infections impairs fertility and on the link between *C. trachomatis* and gonococcal infections and elevated androgen levels.

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interest.

FUNDING

No funding was received for conducting this study.

AUTHOR CONTRIBUTION

AM and KG participated in the conception and design of the study, library searches and assembling relevant literature, critical review of the paper, supervising the writing of the paper, and database management. SJ and AA participated in data collection, library searches and assembling relevant literature, writing the paper, and critical review of the paper. All authors have read and approved the final manuscript.

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