The Association between Infertility and *Helicobacter pylori* Infection: a Meta-Analysis of Case-control Studies

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SUMMARY

Background: *Helicobacter pylori* (*H. pylori*) is increasingly associated with extra-digestive diseases. Infertility is a common condition, with an incidence of 10 to 15% of couples. Studies examining the association of *H. pylori* infection and infertility have reported conflicting results. This meta-analysis aimed to investigate the association between *H. pylori* infection and infertility.

Methods: Studies of *H. pylori* infection and infertility were identified in PubMed, EMBASE, the Cochrane Library, and China National Knowledge Infrastructure. We performed a meta-analysis of all case-control studies.

Results: Seven studies that analyzed the relationship between *H. pylori* infection and infertility, with a combined study population of 1,902 patients, were included in the meta-analysis (n = 626 for patients; n = 1,276 for controls). In the infertility group, 344 (54.9%) patients were *H. pylori*-positive, and 495 (38.8%) were *H. pylori*-positive in the control group. Our result suggested that *H. pylori* infection was associated significantly with infertility (OR = 1.45, 95% CI: 1.197 - 2.160; $I^2$ = 36.5%, Z = 3.15, p = 0.002). Begg’s and Egger’s funnel plot showed no publication bias (p = 0.807).


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KEY WORDS

helicobacter pylori, infertility, meta-analysis

INTRODUCTION

Infertility, a common condition, affects approximately one in seven couples [1,2]. Several factors can result in this condition, and genital tract inflammation caused by pathogens, including *Chlamydia* and *Mycoplasma*, remains a major cause of infertility [3]. Recently, many studies have investigated the role of *Helicobacter pylori* (*H. pylori*) in infertility. IgG released by inflammatory reactions could adhere to sperm, significantly affecting sperm motility and survival [4]. Another possible mechanism by which *H. pylori* could cause infertility is an immune reaction to the sperm antigen [5,6]. *H. pylori* antigens and host cells also promote an immune reaction in the ovary. Meanwhile, anti-*H. pylori* antibodies in the cervical mucus may influence oocyte quality [7].
The composition of cervical mucus, which can be changed by *H. pylori*, is also an essential factor in fertilization. *H. pylori* is correlated with extra-digestive diseases, such as iron-deficiency anemia, vitamin B12 deficiency and idiopathic thrombocytopenic purpura. Meta-analyses of *H. pylori*-related diseases have also been widely reported. Numerous analyses have addressed the role of *H. pylori* in infertility; however, based on a limited sample size, the link between them is weak. Moreover, a meta-analysis of the relationship between *H. pylori* and infertility has not been conducted. Therefore, the aim of our meta-analysis was to investigate the link between *H. pylori* and infertility. Confirmation of the relationship between them will provide evidence for the eradication of *H. pylori* before pregnancy.

**MATERIALS AND METHODS**

**Literature search**

We searched the following databases up to January 2018 and did not restrict our search based on regions, languages, or publication types: PubMed, The Cochrane Library, Excerpta Medica Database (EMBASE), and China National Knowledge Infrastructure (CKNI). The search strategies (Table S1) were performed with both free-text terms and MeSH terms by one of the authors (Xuan Li and Lei Peng).

**Study selection**

First, two investigators (Xiaoran Shen and Jin Yan) independently reviewed the titles and abstracts of all studies. Second, we independently reviewed full texts for further assessment and evaluated whether the articles were suitable using a standardized method. Studies were included if they met the following criteria: i) they examined the association between *H. pylori* infection and infertility and contained sufficient data; and ii) they utilized a case-control, prospective, or cross-sectional study design. Studies were excluded if they satisfied the following exclusion criteria: i) duplicated articles; ii) not clinical trials; iii) not case-control studies; iii) lack of original data after contacting the authors; and iv) case reports, review articles, meta-analyses, letters, commentaries or abstracts presented in conferences. We used Endnote X4 software for article management. Any disagreement was resolved by referring to the other researcher (Guoxin Zhang).

**Data collection and quality assessment**

Data on both study characteristics and research results were retrieved from each study, including the first author, the year of publication, ethnicity, patient characteristics, study design, the causes of infertility, diagnosis method for *H. pylori* infection detection, the number of events in the experimental group and the control group. We evaluated the quality of included studies by the Newcastle-Ottawa quality assessment scale (NOS) [8, 9]. Stars served as a quick visual assessment and were awarded such that the highest quality studies were assigned nine stars, and more than five stars for these studies was optional.

**Statistical analysis**

Meta-analyses were conducted by Stata 14.0 (Stata Corporation, College Station, TX, USA) software. First, when the outcome variables were dichotomous variables, we calculated the odds ratio (OR) with 95% confidence intervals (CIs). According to heterogeneity, the random-effect model was used. Second, the heterogeneity of the trial results was evaluated by the *Q* statistic test and the *I*² measure of inconsistency with a cutoff point of *I*² = 50%. Then, subgroup analysis was performed to identify the source of heterogeneity, and we assessed the consistency of results with sensitivity analyses by omitting one study at a time. Finally, if the data were sufficient, we assessed the publication bias using a funnel plot (p-value lower than 0.05 indicated a significant publication bias) [10-14].

**RESULTS**

**Study selection and study characteristics**

First, a total of 61 articles were obtained by searching the following databases: PubMed (n = 13), the Cochrane Library (n = 9), EMBASE (n = 19), and CKNI (n = 20). Second, we assessed the studies for eligibility and excluded 54 articles using the following criteria: duplicated articles (n = 29), insufficient data (n = 3), commentaries (n = 1), not clinical trials (n = 2), not case-control studies (n = 17), and abstracts presented in conferences (n = 2). Finally, seven articles were selected for our meta-analysis. The flow diagram is shown in Figure 1.

For each study, the data were extracted, and the characteristics are shown in Table 1. A total of 1,902 participants were enrolled, including 626 patients and 1,276 controls. In total, 503 women diagnosed with polycystic ovary syndrome (PCOS) were cases in three articles [15-17]; other women (n = 124) had no PCOS. The design of all included studies was a case-control study. All the included studies detected *H. pylori* IgG antibodies in serum to diagnose *H. pylori* infection. Three articles found no association between *H. pylori* and infertility via serologic examination [16-18]; however, the other studies found that *H. pylori* was a risk factor for infertility [15,17,19].

**Quality assessment**

Table 2 shows the level of evidence provided according to the NOS criteria. A study can be awarded a maximum of one star for each numbered item within the selection and outcome categories. A maximum of two stars can be given for comparability. In the second and third articles, the researchers enrolled in 292 patients with reproductive disorders [20,21], with no description
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Table 1. The characteristics of the included studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Recruitment period</th>
<th>Country</th>
<th>Diagnosis</th>
<th>Study Size (infertility/control)</th>
<th>Mean age (infertility/control)</th>
<th>Infertility group with <em>Hp</em> (+/-)</th>
<th>Control group with <em>Hp</em> (+/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 I Yavasoglu 15</td>
<td>not reported</td>
<td>Japan</td>
<td>PCOS</td>
<td>85 (35/50)</td>
<td>25.0 ± 5.0/26.0 ± 5.0</td>
<td>14/21</td>
<td>11/39</td>
</tr>
<tr>
<td>2 Figura N 20</td>
<td>not reported</td>
<td>Italy</td>
<td>Infertility</td>
<td>1,004 (167/837)</td>
<td>34.4 ± 6.4/not stated</td>
<td>82/85</td>
<td>281/556</td>
</tr>
<tr>
<td>3 Du Jiuwei 21</td>
<td>2003 - 2005</td>
<td>China</td>
<td>Infertility</td>
<td>205 (125/80)</td>
<td>27.6 ± 5.8</td>
<td>58/67</td>
<td>22/58</td>
</tr>
<tr>
<td>4 Wei Qiu 19</td>
<td>2002.5 - 2004.1</td>
<td>China</td>
<td>Infertility</td>
<td>90 (40/50)</td>
<td>34.4 ± 5.0/35.5 ± 4.8</td>
<td>24/16</td>
<td>18/32</td>
</tr>
<tr>
<td>5 AH Kiani 17</td>
<td>2011.6 - 2012.8</td>
<td>Iran</td>
<td>PCOS</td>
<td>254 (127/127)</td>
<td>36.1 ± 3.5/35.8 ± 3.6</td>
<td>79/48</td>
<td>76/51</td>
</tr>
<tr>
<td>6 SG Lou 18</td>
<td>2012.10 - 2013.3</td>
<td>Iran</td>
<td>Infertility</td>
<td>100 (50/50)</td>
<td>21.0 ± 5.8/30.78 ± 5.10</td>
<td>23/27</td>
<td>24/26</td>
</tr>
<tr>
<td>7 F Sohrabvand 16</td>
<td>2010.12 - 2012.5</td>
<td>Iran</td>
<td>PCOS</td>
<td>164 (82/82)</td>
<td>27.2 ± 4.8/27.6 ± 5.2</td>
<td>64/18</td>
<td>63/19</td>
</tr>
</tbody>
</table>

Table 2. Quality assessment of included studies by the Newcastle-Ottawa quality assessment scale (NOS).

<table>
<thead>
<tr>
<th>Study</th>
<th>Selection</th>
<th>Comparability</th>
<th>Exposure</th>
</tr>
</thead>
</table>
|       | Is the case definition adequate | Represen
tativeness of cases | Selection of controls | Definition of controls | Comparability of cases and controls on the basis of the design or analysis | Ascertain of exposure | Same method of ascertainment for cases and controls | Non-Response rate | Scores |
| 1     | *         | *             | *         | *                   | **                              | *                     | *                   | -                  | 8    |
| 2     | -         | *             | *         | *                   | **                              | *                     | *                   | -                  | 7    |
| 3     | -         | *             | *         | *                   | -                               | *                     | *                   | -                  | 5    |
| 4     | *         | *             | *         | *                   | **                              | *                     | *                   | -                  | 8    |
| 5     | *         | *             | *         | *                   | **                              | *                     | *                   | -                  | 8    |
| 6     | *         | *             | *         | *                   | **                              | *                     | *                   | -                  | 8    |
| 7     | *         | *             | *         | *                   | **                              | *                     | *                   | -                  | 8    |

* One star ** Two stars.

or independent validation. For the comparability of cases and controls based on the design or analysis, both the important factor and the other additional factor were not compared between two groups in the third article [21]. None of the included studies described the response rate.

Outcome measure

Data analysis

F. Sohrabvand et al. [16], AK Kiani et al. [17], and SG Lou et al. [18] found that there was no significant difference in *H. pylori* IgG antibodies between the two groups, while the others reported statistically significant findings [15,19-21]. In our meta-analysis, 344/626 patients in the infertility group were positive for *H. pylori* IgG antibodies compared to the control group (495/1,276). Because of low heterogeneity ($I^2 = 36.5\%$, $p = 0.150$), we used the random-effect model for pooling dichotomous data. We found a significant difference in *H. pylori* infection between the two groups (OR = 1.608, 95% CI: 1.197 - 2.160; $Z = 3.15$, $p = 0.002$; Figure 2).

Additional analysis

Subgroup analysis

We also performed subgroup analyses according to region and diagnosis using the random-effects model. Three in seven studies focused on PCOS, which is asso-
Figure 1. Flow diagram of studies identified, included, and excluded.

Figure 2. The Forest plot of the relationship between *H. pylori* infection and infertility, OR with 95% CI.
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**Figure 3. Forest plot of the relationship between *H. pylori* infection and infertility according to the subgroup of diagnosis.**

<table>
<thead>
<tr>
<th>Study ID</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Yavasoglu</td>
<td>2.36 (0.91, 6.12)</td>
<td>7.81</td>
</tr>
<tr>
<td>AH Kiani</td>
<td>1.10 (0.67, 1.83)</td>
<td>18.72</td>
</tr>
<tr>
<td>F. Sornaband</td>
<td>1.07 (0.52, 2.23)</td>
<td>11.66</td>
</tr>
<tr>
<td><strong>Subtotal (I-squared = 56%, p = 0.347)</strong></td>
<td><strong>1.25 (0.84, 1.80)</strong></td>
<td><strong>38.18</strong></td>
</tr>
<tr>
<td><strong>Infertility</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Figura N</td>
<td>1.91 (1.36, 2.67)</td>
<td>26.92</td>
</tr>
<tr>
<td>Du Juwei</td>
<td>2.28 (1.25, 4.17)</td>
<td>15.14</td>
</tr>
<tr>
<td>Wei Qiu</td>
<td>2.67 (1.13, 6.28)</td>
<td>9.23</td>
</tr>
<tr>
<td>S. G. Lou</td>
<td>0.92 (0.42, 2.02)</td>
<td>10.52</td>
</tr>
<tr>
<td><strong>Subtotal (I-squared = 28.5%, p = 0.241)</strong></td>
<td><strong>1.66 (1.31, 2.08)</strong></td>
<td><strong>61.82</strong></td>
</tr>
<tr>
<td><strong>Overall (I-squared = 36.5%, p = 0.150)</strong></td>
<td><strong>1.61 (1.20, 2.16)</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

**Figure 4. Forest plot of the relationship between *H. pylori* infection and infertility according to the subgroup of region.**

<table>
<thead>
<tr>
<th>Study ID</th>
<th>OR (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Yavasoglu</td>
<td>2.36 (0.91, 6.12)</td>
<td>7.81</td>
</tr>
<tr>
<td>Figura N</td>
<td>1.91 (1.36, 2.67)</td>
<td>26.92</td>
</tr>
<tr>
<td><strong>Subtotal (I-squared = 0.0%, p = 0.078)</strong></td>
<td><strong>1.96 (1.42, 2.68)</strong></td>
<td><strong>34.73</strong></td>
</tr>
<tr>
<td><strong>China</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Du Juwei</td>
<td>2.28 (1.25, 4.17)</td>
<td>15.14</td>
</tr>
<tr>
<td>Wei Qiu</td>
<td>2.67 (1.13, 6.28)</td>
<td>9.23</td>
</tr>
<tr>
<td><strong>Subtotal (I-squared = 0.0%, p = 0.771)</strong></td>
<td><strong>2.40 (1.47, 3.94)</strong></td>
<td><strong>24.37</strong></td>
</tr>
<tr>
<td><strong>Iran</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AH Kiani</td>
<td>1.10 (0.67, 1.83)</td>
<td>18.72</td>
</tr>
<tr>
<td>S. G. Lou</td>
<td>0.92 (0.42, 2.02)</td>
<td>10.52</td>
</tr>
<tr>
<td>F. Sornaband</td>
<td>1.07 (0.52, 2.23)</td>
<td>11.66</td>
</tr>
<tr>
<td><strong>Subtotal (I-squared = 0.0%, p = 0.306)</strong></td>
<td><strong>1.96 (0.73, 5.32)</strong></td>
<td><strong>40.90</strong></td>
</tr>
<tr>
<td><strong>Overall (I-squared = 35.5%, p = 0.150)</strong></td>
<td><strong>1.61 (1.20, 2.16)</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

**NOTE:** Weights are from random effects analysis.
Figure 5. Sensitivity analysis of this meta-analysis.

In this meta-analysis, we identified a relationship between H. pylori infection and infertility. A total of seven case-control studies up to April 2017 were included, involving 626 patients and 1,276 controls. The positive status of H. pylori infection was defined by enzyme-linked immunosorbent assay (ELISA) of serum H. pylori IgG antibody. The rate of H. pylori infection in infertility patients was 54.9%, whereas the rate was 38.8% in the control group. These results suggested that H. pylori infection was increased in the infertility group.

Infertility, which is observed in 10 to 15% of couples (aged 15 - 44 years), is a social problem [22]. Genital tract infertility is widely considered to be an important problem and can be caused by infectious agents, such as Neisseria gonorrhoea, Chlamydia trachomatis, Enterobacteria, Gram-positive cocci, HIV-1, and H. pylori. An antigen produced by human spermatozoa shows immune cross-reactions with yeasts or bacteria such as Herpesvirus hominis or HIV-1. Infertility may also result from the salpingitis and epididymitis due to chlamydia [23].

Yavasoglu et al. [15] found that anti-H. pylori antibodies in the cervical mucus were correlated with those in the serum. Ambrosini et al. [24] also measured anti-H. pylori antibodies in cervical mucus and showed a significant positive association between infertility and H.
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The *Helicobacter pylori* infection (p < 0.00001). Unfortunately, due to the limitation of original data, this article was excluded in our meta-analysis. The mechanisms underlying the association with infertility in women may include a negative effect of ovarian follicular fluid on oocyte quality. Du Jiuwei et al. [21] analyzed the levels of CD4⁺, CD8⁺, IL-4, IL-8, IFN-γ, and TNF-α. The results showed *H. pylori* infection increased the levels of IL-4, IL-8, IFN-γ, and TNF-α and decreased the levels of CD4⁺, CD8⁺, and CD4⁺/CD8⁺. These findings indicate dysfunctional T-lymphocyte activation and enhanced humoral immunity. Inflammatory factors, such as TNF-
α and IFN-γ, reduced apoptosis of human trophoblast cells in early pregnancy and were associated with infertility. Meanwhile, cytokines released by *H. pylori* may cause autoimmune responses that damage the integrity of the fetoplacental unit and threaten the welfare of the fetus [25].

In addition to infertility, many studies have shown that *H. pylori* is associated with other gynecological diseases, such as hyperemesis gravidarum [26] and pre-eclampsia [27]. The role of *H. pylori* in various extra-digestive diseases, such as endocrine system disease (type 2 diabetes), also has to be investigated [28]. In *vivo*, researchers found that *H. pylori* colonization levels could be affected by female hormones [29]. PCOS is a heterogeneous disorder characterized by infertility [29]. Women with PCOS may have an increased risk of type 2 diabetes [28,30]. The mechanisms underlying the associations among *H. pylori* infection, hormones, and infertility may be related to an immune cross-reaction. Vasoactive substances, such as eicosanoids and cytokines, are produced in response to *H. pylori* infection, and then, substances may be transported through blood circulation and promote an immune cross-reaction in ovary. In male patients, Figura N et al. [20] found a reaction between a serum raised to a *H. pylori* whole-cell suspension and human spermatozoa by immunofluorescence. They also found partial linear homology between human tubulin and *H. pylori* flagellin, CagA and VacA. As a hypothetical mechanism, *H. pylori* infection, which is associated with low sperm quality, may influence the composition of spermatozoa antigens through systemic inflammation [4,31]. Therefore, the capacity for sperm-oocyte fusion could be reduced by these agents [32]. We also conducted subgroup analyses based on diagnoses and region. With regard to diagnosis, we found that the results for PCOS were not significant. Patients suffering from syndromes potentially associated with infertility may influence final results. For subgroup analyses of different regions, the relationship between *H. pylori* infection and infertility was not significant in Iran, in contrast to this relationship in other countries. Additionally, the heterogeneity in separated sub-groups was not significant.

Some limitations of this meta-analysis should be acknowledged. First, the quality of included case-control studies was not as high as that of the randomized clinical trials. Second, the included articles were published up to April 2017, and unpublished studies may have been missed. Third, despite no significant publication bias, it is also possible that related studies with negative results might be unpublished, resulting in an unavoidable publication bias. Finally, in the seven included reports, three studies enrolled women with PCOS [15-17], while patients were diagnosed with infertility in other studies [18-21]. Two articles involved men with infertility [20,21]. There was a small sample size in the study of men with infertility. We strongly recommend that additional studies with large sample sizes, male infertility patients, and past and active infection conditions should be conducted to assess the relationship between *H. pylori* infection and infertility.

**CONCLUSION**

In conclusion, this meta-analysis found increased *H. pylori* seropositivity in the infertility group. We hypothesize that there may be a possible relationship between *H. pylori* infection and infertility, indicating that *H. pylori* may be a risk factor for infertility. In addition, further studies are required to evaluate more subjects and identify the mechanism of *H. pylori* infection and infertility.

**Acknowledgment:**

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**Author Contributions:**

Xuan Li and Guoxin Zhang conceived and designed the experiments; Lei Peng, Jin Yan and Shenxiao Ran performed the experiments and analyzed the data; Xuan Li wrote the paper.

**Supporting Information:**

Table S1 - Construction of search strategies: EMBASE: Excerpta Medica Database; CNKI: China National Knowledge Infrastructure; MeSH: Medical Subject Heading.

**Declaration of Interest:**

There is no conflict of interest.

**References:**

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Additional material can be found online at: http://supplementary.clin-lab-publications.com/180206/