Is *Chlamydia trachomatis* PCR Detection from Cervical Canal Swabs Associated with Tubal Obstruction?

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ABSTRACT

Objective: To determine the relationship between a *Chlamydia trachomatis* PCR positive diagnosis from cervical canal swabs and the presence of tubal diseases among infertile women in Vietnam.

Methods: In this cross-sectional descriptive study, women who sought infertility treatment at the Center for Reproductive Endocrinology & Infertility, Hue University Hospital, Vietnam, from June 2016 to June 2017 were enrolled. All study participants were interviewed, and PCR tests were then performed to diagnose *Chlamydia* from cervical canal swabs. Hysterosalpingogram (HSG) was carried out to examine the uterine cavity and fallopian tubes.

Results: Among 568 women whose mean age was 32.0 ± 5.1 years, the prevalence of *C. trachomatis* infection as detected by PCR was 5.8%. Eighty-one percent (460/568) of infertile women had normal HSG results, and abnormal HSG results were more frequent in women over 35 years old, in women with secondary infertility, and in those with a history of miscarriage or genital tract infection. However, there was no relationship between *C. trachomatis* PCR positivity and HSG results in infertile women.

Conclusions: The diagnosis of *C. trachomatis* infection using the cervical swabs is the useful but not an effective method for routine practice for predicting tubal obstruction in infertile women.

Keywords: *Chlamydia trachomatis*; Infertility; Cervical Swab; Tubal Disorders.

TÓM TẮT
(ABSTRACT IN VIETNAMESE)

Mục tiêu: Xác định mối quan hệ giữa chẩn đoán *Chlamydia trachomatis* PCR dương tính từ bệnh phẩm ống cổ tử cung và bệnh lý vòi tử cung ở phụ nữ vô sinh ở Việt Nam.

Phương pháp: Trong nghiên cứu mô tả cắt ngang này, những phụ nữ đến điều trị vô sinh tại Trung tâm Nội tiết sinh sản & Vô sinh, Bệnh viện Đại học Y Dược Huế, Việt Nam, từ tháng 6 năm 2016 đến tháng 6 năm 2017 đã được nhận vào mẫu nghiên cứu. Tất cả những người tham gia nghiên cứu đã được phỏng vấn, khám phụ khoa và xét nghiệm PCR chẩn đoán *C. trachomatis* từ bệnh phẩm dịch ống cổ tử cung. Chụp phim tử cung – vòi tử cung có bơm chất cản quang (HSG) được thực hiện để kiểm tra buồng tử cung và vòi tử cung.

Kết quả: Tổng số 568 phụ nữ được nghiên cứu có tuổi trung bình là 32,0 ± 5,1 tuổi, tỷ lệ nhiễm *C. trachomatis* được phát hiện bằng PCR là 5,8%. Tám mươi mốt phần trăm (460/568) phụ nữ vô sinh có kết quả HSG bình thường, và kết quả HSG bất thường xảy ra nhiều hơn ở phụ nữ trên 35 tuổi, ở phụ nữ vô sinh thứ phát và ở những người có tiền sử sảy thai hoặc nhiễm trùng đường sinh dục. Tuy nhiên, không có mối quan hệ giữa kết quả dương tính với *C. trachomatis* PCR và bất thường HSG ở phụ nữ vô sinh.

Kết luận: Chẩn đoán nhiễm *C. trachomatis* từ dịch ống cổ tử cung là phương pháp hữu ích nhưng không phải là phương pháp hiệu quả để thực hành thường quy trong dự đoán bệnh lý vòi tử cung ở phụ nữ vô sinh.

Từ khóa: *Chlamydia trachomatis*; dịch ống cổ tử cung; bệnh lý vòi tử cung.

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INTRODUCTION

Infertility, which is defined as the inability to conceive after 12 months of regular unprotected sexual intercourse, affects approximately 9%–12% of reproductive-aged women worldwide (Barbara et al., 2016; Datta et al., 2016). The inability to conceive results not only in a considerable financial burden on patients and the healthcare system but also in psychological stress for millions of couples (Cousineau and Alice, 2006). Tubal disease is among the most common causes of infertility and is the primary diagnosis in approximately 30% of female infertility cases (Chaudhari et al., 2017).

Pelvic inflammatory disease (PID) is the single most important cause of tubal pathology leading to infertility. The two organisms that are mostly frequently related to upper genital tract infections (GTIs) are *Neisseria gonorrhea* and *Chlamydia trachomatis* (Paavonen and Eggert-Kruse, 1999). *Chlamydia* is now recognized to be associated with at least 50% of cases of acute PID in developed countries and occurs in 50%–80% of female patients. Unfortunately, because *C. trachomatis* infections are asymptomatic in most women, infections are often unnoticed, untreated, and not reported, it is challenging to diagnose tubal disease based on the early detection of *C. trachomatis* (Papp et al., 2014; Zakher et al., 2014). The recommendation is to screen this group of patients for *Chlamydia* infection to prevent the sequelae of PID with severe tubal damage (Thomas et al., 2000).

C. trachomatis is an obligate intracellular Gram-negative bacterium and is one of the most common bacterial sexually transmitted infections (STIs) (Manavi, 2006). It is estimated that every year, approximately 92 million new cases of *C. trachomatis* infection occur worldwide (Gaydos et al., 2004). Although *Chlamydia* infection is a preventable cause of infertility, the remarkable worldwide increase in the incidence of PID during recent decades has led to increasing rates of primary and secondary tubal factor infertility (Malik et al., 2009).

Hysterosalpingography (HSG) is the most commonly used method of assessment for tubal conditions as an initial investigation because it is cheaper and less invasive (Dabekausen et al., 1994; Swart et al., 1995). It has been reported that 73%–79% of infertile women with tubal abnormalities by HSG are positive for *C. trachomatis* antibody (Moore et al., 1982). Tuboperitoneal abnormalities have been observed in 81.4% of *Chlamydia* seropositive patients versus 13.2% of seronegative patients. In women with tubal damage, the frequency of positive serum *Chlamydia* antibody titers (CATs) (≥1:32) was significantly higher than that in women who had a normal pelvic peritoneum (66.6% vs. 6.5%, $p < 0.001$) (Peivandi et al., 2009). In a previous study, a significant association was found between *C. trachomatis* IgG, *C. trachomatis* PCR positivity and tubal factor infertility and showed that *C. trachomatis* infection is an important cause of PID and infertility (Deshmukh, 2013). Logically, *C. trachomatis* infection should be a marker of tubal disease or poor prognosis for fertility. However, in a study by Muvunhi et al., the rate of *C. trachomatis* infection as detected by PCR in infertile groups was 3.3% (12/312), which did not differ significantly from that in fertile groups (Muvunyi et al., 2011). In practice, PCR testing is the gold standard for the direct detection of Chlamydia because other methods, such as isolation by cell culture and antigen detection, are less specific and sensitive (Meyer, 2016). The question is whether the presence of *C. trachomatis*, as diagnosed from cervical canal swabs as the routine practice, is associated with tubal disease. The present study aimed to determine the prevalence of *C. trachomatis* PCR positivity and its association with tubal diseases among infertile women in Vietnam.

MATERIALS AND METHODS

In this cross-sectional descriptive study, women who sought infertility treatment at the Center for Reproductive Endocrinology & Infertility, Hue University Hospital, Vietnam from June 2016 to June 2017 were recruited. The exclusion criteria were menorrhagia, treatment of GTI with systematic or local antibiotics within 4 weeks before inclusion or refusal to be enrolled in the study population.

All women were interviewed according to a prepared protocol containing administrative data, the obstetrics and gynecology history, and the GTI and previous management history. A standardized physical and pelvic examination was then carried out, which included questions regarding vulvovaginal problems; inspections of the vulva, vagina, and cervix; measurements of vaginal pH and microscopic inspections by vaginal wet mount and Gram staining. All women were screened for *Chlamydia* infection in their cervical discharge by real-time PCR.

Each subject was measured for height and weight. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. Based on the Asian-specific classification for BMI status, BMI values were categorized as underweight (<18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥30 kg/m^2). We defined oligomenorrhea as having fewer than 8 menstrual cycles per year, the absence of 3 to 6 consecutive menstrual cycles per year or intermenstrual intervals ≥35 days. The normal menstrual cycle lasted from 24 to 35 days.

Real-time PCR for detecting *Chlamydia*: The ^{iVA}pDNA Extraction Kit (Viet A Technology Corp., HCM, Vietnam) was used for DNA extraction from vaginal swab samples as recommended by the manufacturer, with 10 µL of internal control. Real-time PCR assays were performed using a forward primer (5′-CATGAAAACTCGTTCCGAAATAGAA-3′), a reverse primer (5′-TCAGAGCTTTACCTAACAACGCATA-3′), and the TaqMan probe 5′-FAM (5′-TCGCATG-CAAGATATCGA-3′), which specifically targeted the 71-bp DNA segment of the cryptic plasmid of *C. trachomatis* (Jaton et al., 2006). The reactions were performed in a final volume of 25 µL, including 0.2 µM each primer, 0.1 µM probe, 12.5 µL of 2 × TaqMan Universal Master Mix (Applied Biosystems, USA), and 5 µL of DNA sample. PCR amplification was performed as follows: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 15 s, 55°C for 60 s, and 72°C for 20 s, in the Mx3000P qPCR System (Agilent Technologies Inc., CA, USA). The result was analyzed with MxPro QPCR Software (Agilent Technologies Inc., CA, USA). The real-time PCR outcome was considered positive if the negative controls were all negative and if the FAM signal was equal to a cycle threshold value (Ct) of ≤35.

Hysterosalpingogram (HSG) studies were evaluated by experienced practitioners in all cases to examine the cavity of the uterus and fallopian tubes. First, a control image was obtained before the injection of contrast (Ultravist 300 (iopromide), Bayer, UK). Then, approximately 10–20 mL of contrast was injected in each case, and a minimum of 3 additional images were obtained to examine the uterine cavity, tubal patency and the presence of contrast medium in the pelvic cavity.

All analyses were performed using the Statistical Product and Service Solutions (SPSS) version 20.0 (SPSS Inc., Chicago, IL, USA). Continuous variables between groups were compared using the independent samples t-test for normally distributed data or the Mann–Whitney U-test for skewed data. For comparisons of categorical variables, we used the chi-square (χ^2) test or Fisher's exact test, where appropriate. The results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs) or two-sided p-values. A p-value of <0.05 was considered statistically significant. The study

was approved by the Hue University of Medicine and Pharmacy Ethics Committee.

RESULTS

A total of 568 women who underwent infertility investigations and treatment agreed to participate in our study. The mean age of the women was 32.0 ± 5.1 years. The duration of infertility ranged from 1 to 22 years (mean ± SD: 3.7 ± 2.9). The proportions of primary and secondary infertility cases were 365/568 (64.3%) and 203/568 (35.7%), respectively, as shown in Table 1.

Table 1 also shows that the rate of *C. trachomatis* infection as detected by PCR was 5.8% (33/568). The mean age of the infertile women with PCR *C. trachomatis* positivity was lower than that of the women who were negative (29.9 ± 5.2 vs. 32.1 ± 5.1 years), but there was no statistically significant difference. The PCR-positive rate was higher for primary infertility than for secondary infertility, but the difference was not statistically significant (7.1% and 3.4%, $p > 0.05$).

As shown in Table 2, 81.0% (460/568) of infertile women had normal HSG results. Nineteen percent of infertile women had abnormal HSG results, and of these women, 7.9% had tubal

Table 2. Hysterosalpingography results of the infertile population.

Hysterosalpingography results	N	%
Patent	460	81.0
Occlusion 1 side	45	7.9
Occlusion 2 sides	35	6.2
Hydrosalpinx 1 side	20	3.5
Hydrosalpinx 2 sides	8	1.4
Total	568	100

Table 1. General characteristics of the study population and *C. trachomatis* PCR detection from cervical canal swabs.

Characteristics \ *C. trachomatis*		Infection (n = 33)		Non-infection (n = 535)		Total n = 568		p-Value
		N	%	N	%	N	%	
Age	≤35	27	6.3	404	93.7	431	75.9	0.411
	>35	6	4.4	131	95.6	137	24.1	
	Mean ± SD	29.9 ± 5.2		32.1 ± 5.1		32.0 ± 5.1		
Occupation	Office work	19	6.3	285	93.7	304	5.35	0.740
	Manual work	5	3.6	135	96.4	140	24.6	
	Business	3	7.0	40	93.0	43	7.6	
	Others	6	7.4	75	92.9	81	14.3	
Geography	Urban	10	4.0	239	96.0	249	43.8	0.106
	Nonurban	23	7.2	296	92.8	319	56.2	
Education	School grade	12	5.0	226	95.0	238	41.9	0.506
	College/ University grade	21	6.4	309	93.6	330	58.1	
Infertility type	Primary	26	7.1	339	92.9	365	64.3	0.073
	Secondary	7	3.4	196	96.6	203	35.7	
Infertility duration	≤3 years	27	8.1	305	91.9	332	58.5	0.005
	>3 years	6	2.5	230	97.5	236	41.5	
	Mean ± SD	2.2 ± 1.6		3.8 ± 2.9		3.7 ± 2.9		
History of miscarriage	Yes	2	1.6	125	98.4	127	22.4	0.021
	No	31	7.0	410	93.0	441	77.6	
History of GTI	Yes	1	1.6	63	98.4	64	11.3	0.159
	No	32	6.3	472	93.7	504	88.7	
Menstrual cycle	Irregular	13	6.1	199	93.9	212	37.2	0.800
	Regular	20	5.6	336	94.4	356	62.8	
BMI	<18.5	15	16.7	75	83.3	90	15.8	<0.001
	18.5–22.9	15	3.8	379	96.2	394	69.4	
	≥23.0	3	3.6	81	96.4	84	14.8	
	Mean ± SD	19.6 ± 2.7		20.7 ± 2.3		20.7 ± 2.4		
Oligo/Anovulation	Yes	5	4.5	107	95.5	112	19.7	0.497
	No	28	6.1	428	93.9	456	80.3	

C. trachomatis: *Chlamydia trachomatis*, GTI: genital tract infection, BMI: body mass index.

Table 3. General characteristics of the study population and the hysterosalpingography results.

Characteristics \ HSG result		Abnormal (n = 108)		Normal (n = 460)		Total n = 568		p-Value
		N	%	N	%	N	%	
Age	≤35	70	16.2	361	83.8	431	75.9	0.003
	>35	38	27.7	99	72.3	137	24.1	
	Mean ± SD	33.3 ± 5.4		31.7 ± 5.0		32.0 ± 5.1		
Occupation	Office work	58	19.1	246	80.9	304	53.5	0.753
	Manual work	30	21.4	110	78.6	140	24.6	
	Business	7	16.2	36	83.8	43	7.6	
	Others	13	16.0	68	84.0	81	14.3	
Geography	Urban	50	20.1	199	79.9	249	43.8	0.567
	Nonurban	58	18.2	261	81.8	319	56.2	
Education	School grade	44	18.5	194	81.5	238	100	0.786
	College/University grade	64	19.4	266	80.6	330	100	
Infertility type	Primary	45	12.3	320	87.7	365	64.3	<0.001
	Secondary	63	31.0	140	69.0	203	35.7	
Infertility duration	≤3 years	45	13.6	287	86.4	332	58.5	<0.001
	>3 years	63	26.7	173	73.3	236	41.5	
	Mean ± SD	4.6 ± 3.2		3.5 ± 2.8		3.7 ± 2.9		
History of miscarriage	Yes	40	31.5	87	68.5	127	22.4	<0.001
	No	68	15.4	373	84.6	441	77.6	
History of GTI	Yes	21	32.8	43	67.2	64	11.3	0.003
	No	87	17.3	417	82.7	504	88.7	
Menstrual cycle	Irregular	31	14.6	181	85.4	212	37.3	0.040
	Regular	77	21.6	279	78.4	356	62.7	
BMI	<18.5	17	18.9	73	81.1	90	15.8	0.953
	18.5–22.9	74	18.8	320	81.2	394	69.4	
	≥23.0	17	20.2	67	79.8	84	14.8	
	Mean ± SD	20.7 ± 2.3		20.7 ± 2.4		20.7 ± 2.4		
Oligo/Anovulation	Yes	16	14.3	96	85.7	112	19.7	0.155
	No	92	20.2	364	79.8	456	80.3	

GTI: genital tract infection, BMI: body mass index.

occlusion on one side, 6.2% had tubal occlusions on two sides, 1.4% had tubal hydrosalpinx on two sides (hydrosalpinx — fallopian tube that is blocked with aqueous fluid), and 3.5% had hydrosalpinx on one side.

Table 3 shows that the mean age of the women with abnormal HSG results was higher than that of the women with normal HSG results (33.3 ± 5.4 and 31.7 ± 5.0). Women over 35 years old had a high abnormal HSG rate compared to women ≤ 35 years old (27.7% and 16.2%, respectively; $p < 0.05$).

The women with primary infertility had a lower rate of abnormal HSG than the women with secondary infertility (12.3% and 31.0%, respectively; $p < 0.05$).

The rate of abnormal HSG increased as the infertility duration increased. The mean infertility duration of the women with abnormal HSG was higher than that of the women with normal HSG (4.6 ± 3.2 and 3.5 ± 2.8, respectively; $p < 0.05$).

These data indicated that there was no relationship between the abnormal HSG rate and several of the characteristics of the infertile women, such as occupation, geography, education, and BMI.

Women with a history of miscarriage or GTI had significantly higher abnormal HSG rates.

Table 4. Value of *C. trachomatis* PCR detection from cervical swabs in predicting the HSG result.

C. trachomatis \ HSG	Abnormal		Normal		P
	N	%	N	%	
Positive	7	6.5	26	5.7	
Negative	101	93.5	434	94.3	0.819
Total	108	100	460	100	

C. trachomatis: *Chlamydia trachomatis*, HSG: Hysterosalpingography.

As shown in Table 4, there was no relationship between *C. trachomatis* PCR positivity and the HSG results in infertile women ($p > 0.05$), with a rate of *C. trachomatis* infection that was comparable between normal and abnormal HSG results (5.7% vs. 6.5%).

DISCUSSION

In our study, the overall prevalence of *C. trachomatis* as determined by direct PCR was 5.8%. In previous studies of the same subjects

and with the same method of *Chlamydia* detection (by direct PCR) from cervical discharge (vaginal swabs), the rates of *C. trachomatis* infection were 3.2% (Muvunyi et al., 2011), 5% (Joolayi et al., 2017), 6.8% (Idahl et al., 2004), and 3.9% (Al Ramahi et al., 2008). However, other studies reported rates for *C. trachomatis* of up to 20.2% (El Qouqa et al., 2009) and 13.5% (Dhawan et al., 2014). In particular, in studies in which *Chlamydia* was detected by serum antibody testing, the rates of *C. trachomatis* infection were much higher, at 24.2% in A. Idahl's study (Idahl et al., 2004) or 15% in Svenstrup's study (Svenstrup et al., 2008).

Currently, various diagnostic methods, such as isolation in cell culture, antigen detection methods, and nucleic acid amplification tests (NAATs) for the direct detection of *C. trachomatis*, are available. Of these methods, NAATs (PCR) are the most attractive diagnostic tools for screening asymptomatic individuals because of their sensitivity and specificity and their noninvasive use with collected specimens (Johnson et al., 2002; Zakher et al., 2014). Although *C. trachomatis* IgG positivity points to the cause of tubal infertility in infertile women, the results of direct detection methods are almost always negative in these women. The problem is that *Chlamydia* serological examinations cannot differentiate between an acute and a previous *Chlamydia* infection since IgG persists for many years in most women after successful treatment. Therefore, cervical swabs for *Chlamydia* infection tests remain routine in examining women for gynecological problems.

Most studies agree that women who have more than one sexual partner are more at risk than those who do not frequently change partners. Additionally, low education seems to be another risk factor (Walker et al., 2012). However, these data indicate that there is no relationship between *C. trachomatis* infection and several of the characteristics of the infertile women.

Tubal damage as the etiology accounts for 18% of infertility cases (Althaus et al., 2012). The etiology of tubal damage can be intrinsic (ascending salpingitis, including salpingitis isthmica nodosa) or extrinsic (peritonitis, endometriosis, or pelvic surgery) (Althaus et al., 2012). Studies have demonstrated that the severity of tubal damage found in infertile women is directly related to their serum *Chlamydia* antibody IgG titer (CAT) (Akande et al., 2010; Althaus et al., 2012). Hysterosalpingography (HSG) is the most commonly used method of assessment for tubal disease as an initial investigation because it is cheaper and less invasive (Dabekausen et al., 1994; Swart et al., 1995). Tubal abnormalities that can be detected include tubal occlusion, salpingitis isthmica nodosum, polyps, hydrosalpinx, and peritubal adhesions (Althaus et al., 2012). Among 568 women who underwent HSG in this study, 460 women (81%) had patent tubes with bilateral spillage. This result was similar to that of Kumari's study (84%) (Kumari et al., 2017).

Infection with *C. trachomatis* results in the generation of antibodies that are detectable in the serum. Studies using laparoscopy have confirmed that serological evidence of past infection with *C. trachomatis* is associated with a significantly increased risk of tubal infertility in women (Akande et al., 2003; Coppus et al., 2007). Currently, PCR is the most attractive diagnostic tool for screening *Chlamydia* because of its sensitivity, specificity, and noninvasiveness (Johnson et al., 2002; Zakher et al., 2014). There is not much evidence of a relationship between the *Chlamydia* screening results by PCR and the results of HSG in terms of a finding of tubal abnormality. However, in examining the present data, we found no relationship between the results of *C. trachomatis* PCR and HSG in infertile women ($p > 0.05$), and it once again confirms the uselessness of cervical swab testing of *C. trachomatis* for the prediction of tubal disorders. A limitation of this study was without information on laparoscopic findings, which would help to confirm whether there was genuine tubal pathology and what was the underlying pathology to account for the abnormal HSG findings (endometriosis, previous surgery, etc.) in the study population. Therefore, HSG could only determine the tubal obstruction.

In conclusion, the diagnosis of *C. trachomatis* infection using the cervical swaps is the useful but not an effective method for routine practice for predicting tubal obstruction in infertile women.

COMPETING INTERESTS

The authors have no competing financial or other interests to declare in relation to this manuscript.

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REVIEW BOARD STATUS

The study was approved by the Hue University of Medicine and Pharmacy Ethics Committee.

AUTHORS' CONTRIBUTIONS

L.M.T, N.V.Q.H, and N.T.L.N participated in the study design, data collection, data analysis, manuscript drafting, and critical discussion. N.V.Q.T, N.H.B, and C.N.T participated in the study design, interpretation of data and critical discussion. All authors have been involved in drafting the work or revising it critically for important intellectual content in the final manuscript.

REFERENCES

Akande V, Turner C, Horner P, Horne A, Pacey A. Impact of *Chlamydia trachomatis* in the reproductive setting: British fertility society guidelines for practice. Hum Fertil. 2010;13(3):115–25. doi:10.3109/14647273.2010.513893

Akande VA, Hunt LP, Cahill DJ, Caul EO, Ford WC, Jenkins JM. Tubal damage in infertile women: prediction using *Chlamydia* serology. Hum Reprod. 2003;18(9):1841–7. doi:10.1093/humrep/deg347

Al Ramahi M, Mahafzah A, Saleh S, Fram K. Prevalence of *Chlamydia trachomatis* infection in infertile women at a university hospital in Jordan. East Mediterr Health J. 2008;14(5):1148–54.

Althaus CL, Heijne JC, Herzog SA, Roellin A, Low N. Individual and population level effects of partner notification for *Chlamydia trachomatis*. PLoS ONE. 2012;7(12):e51438. doi:10.1371/journal.pone.0051438

Barbara LH, John OS, Karen DB, Lisa MH, Joseph IS, Marlene MC. Evaluation of the Infertile Couple. Williams Gynecology. 3rd ed. New York: McGraw-Hill; 2016: pp. 427–48.

Chaudhari AD, Baria AM, Chaudhari UR. Diagnostic laparoscopy in the evaluation of tubal factor in cases of infertility. Int J Reprod Contracept Obstet Gynecol. 2017;6(4):1275–8. doi:10.18203/2320-1770.ijrcog20171032

Coppus SF, Opmeer BC, Logan S, Van der Veen F, Bhattacharya S, Mol BW. The predictive value of medical history taking and *Chlamydia* IgG ELISA antibody testing (CAT) in the selection of subfertile women for diagnostic laparoscopy: a clinical prediction model approach. Hum Reprod. 2007;22:1353–8.

Cousineau TMD, Alice D. Psychological impact of infertility. Clin Obstet Gynecol. 2006;21:293–308.

Dabekausen YA, Evers JL, Land JA, Stals FS. *Chlamydia trachomatis* antibody testing is more accurate than hysterosalpingogram in

predicting tubal factor infertility. Fertil Steril. 1994;61(5): 833–7.

Datta J, Palmer MJ, Tanton C, et al. Prevalence of infertility and help seeking among 15000 women and men. Hum Reprod. 2016;31(9):2108–18. doi:10.1093/humrep/dew123

Deshmukh DG. Study of *Chlamydia trachomatis* in infertile women. Paripex Indian J Res. 2013;2(3):260–3.

Dhawan B, Rawre J, Ghosh A, et al. Diagnostic efficacy of a real time-PCR assay for *Chlamydia trachomatis* infection in infertile women in North India. Indian J Med Res. 2014;140(2):252–61.

El Qouqa IA, Shubair ME, Al Jarousha AM, Sharif FA. Prevalence of *Chlamydia trachomatis* among women attending gynecology and infertility clinics in Gaza, Palestine. Int J Infect Dis. 2009;13(3):334–41. doi:10.1016/j.ijid.2008.07.013

Gaydos CA, Theodore M, Dalesio N, Wood BJ, Quinn TC. Comparison of three nucleic acid amplification tests for detection of *Chlamydia trachomatis* in urine specimens. J Clin Microbiol. 2004;42:3041–5. doi:10.1128/JCM.42.7.3041–3045.2004

Idahl A, Boman J, Kumlin U, Olofsson JI. Demonstration of *Chlamydia trachomatis* IgG antibodies in the male partner of the infertile couple is correlated with a reduced likelihood of achieving pregnancy. Human Reprod. 2004;19(5):1121–6. doi:10.1093/humrep/deh155

Jaton K, Bille J, Greub G. A novel real-time PCR to detect *Chlamydia trachomatis* in first-void urine or genital swabs. J Med Microbiol. 2006;55:1667–74. doi:10.1099/jmm.0.46675-0

Johnson RE, Newhall WJ, Papp JR, et al. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections. MMWR Recomm Rep. 2002;51(RR-15):1–38.

Joolayi F, Navidifar T, Jaafari RM, Amin M. Comparison of *Chlamydia trachomatis* infection among infertile and fertile women in Ahvaz, Iran: a case control study. Int J Reprod BioMed. 2017;15(11):713–8.

Kumari TM, Swetha A, Sangabathula H. A study on role of hysterosalpingogram (HSG) in evaluation of female infertility. Int J Contemp Med Surg Radiol. 2017;2(4):164–6.

Malik A, Jain S, Rizvi M, Shukla I, Hakim S. *Chlamydia trachomatis* infection in women with secondary infertility. Fertil Steril. 2009;91(1):91–5. doi:10.1016/j.fertnstert.2007.05.070

Manavi K. A review on infection with *Chlamydia trachomatis*. Best Pract Res Clin Obstet Gynaecol. 2006;20(6):941–51. doi:10.1016/j.bpobgyn.2006.06.003

Meyer T. Diagnostic procedures to detect *Chlamydia trachomatis* infections. Microorganisms. 2016;4:25. doi:10.3390/microorganisms4030025

Moore D, Foy H, Daling J, et al. Increased frequency of serum antibodies to *Chlamydia trachomatis* in infertility due to distal tubal disease. Lancet. 1982;2:574–7.

Muvunyi CM, Dhont N, Verhelst R, Temmerman M, Claeys G, Padalko E. *Chlamydia trachomatis* infection in fertile and subfertile women in Rwanda: prevalence and diagnostic significance of IgG and IgA antibodies testing. Hum Reprod. 2011;26(12):3319–26. doi:10.1093/humrep/der350

Paavonen J, Eggert-Kruse W. *Chlamydia trachomatis*: impact on human reproduction. Hum Reprod. 1999;5(5):433–47.

Papp JR, Schachter J, Van Der Pol B. Recommendations for the laboratory-based detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. MMWR Recomm Rep. 2014;63: 1–19.

Peivandi S, Moslemizadeh N, Gharajeh S, Ajami A. The role of *Chlamydia trachomatis* IgG antibody testing in predicting tubal factor infertility in Northern Iran. Int J Fertil Steril. 2009;3(3): 143–8.

Svenstrup HF, Fedder J, Kristoffersen SE, Trolle B, Birkelund S, Christiansen G. *Mycoplasma genitalium*, *Chlamydia trachomatis* and tubal factor infertility — a prospective study. Fertil Steril. 2008;90(3):513–20. doi:10.1016/j.fertnstert.2006.12.056

Swart P, Mol BW, van der Veen F, van Beurden M, Redekop WK, Bossuyt PM. The accuracy of hysterosalpingography in the diagnosis of tubal pathology: a meta-analysis. Fertil Steril. 1995;64(3):486–91.

Thomas K, Coughlin L, Mannion PT, Haddad NG. The value of *Chlamydia trachomatis* antibody testing as part of routine infertility investigations. Hum Reprod. 2000;15(5):1079–82.

Walker J, Tabrizi SN, Fairley CK, et al. *Chlamydia trachomatis* incidence and re-infection among young women — behavioural and microbiological characteristics. PLoS One. 2012;7(5): e37778.

Zakher B, Cantor AG, Pappas M, Daeges M, Nelson HD. Screening for *Gonorrhea* and *Chlamydia*: systematic review to update the U.S. Preventive Services Task Force Recommendations. Evidence Synthesis. 2014;No115.