

## ***Chlamydia trachomatis* C-complex Serovars are a Risk Factor for Preterm Birth**

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**Abstract.** *Background: Potential associations between current or previous C. trachomatis infections (general IgG and serovars) and spontaneous preterm birth (PTB) were examined and associations between C. trachomatis infections and previous fertility problems were explored. Patients and Methods: At week 17, Chlamydia antigen, Chlamydia IgG, Chlamydia complex B, C and GF levels were measured. Spontaneous preterm birth (<37 completed weeks gestation) was the primary outcome, and secondary outcomes included infertility treatment, subfertility and ectopic pregnancies. Crude and adjusted relative risks (RR) and odds ratios (OR) were estimated by logistic regression. Results: C. trachomatis C-complex positivity was associated with spontaneous PTB [RR 2.6(1.1-6.29)] and additionally with a prior history of subfertility [OR 4.4(2.5-7.7)], infertility treatment [OR 7.2(4.0-12.8)] and ectopic pregnancy [5.2(2.2-12.4)]. Conclusion: A previous infection with C. trachomatis C-complex was associated with an increased risk for spontaneous PTB and may potentially contribute to subfertility, infertility and ectopic pregnancy.*

*Chlamydia trachomatis* is the most prevalent bacterial sexually transmitted disease. According to the World Health Organization, approximately 90 million *C. trachomatis* infections are annually detected worldwide (1). The infection is estimated to be asymptomatic in 50% of males and in up to 80% of females (2) and is found in close to 90% of the infertile women (3). The morbidity related to sexually transmitted *C. trachomatis* infections is severe and *C. trachomatis* antibody IgG and IgM were associated with

preterm birth (PTB), ectopic pregnancy, subfertility, tubal infertility, spontaneous abortion, premature rupture of membranes and neonatal infections (4-6). As a *C. trachomatis* infection is a preventable cause of both infertility and adverse pregnancy outcome (3, 7), effective diagnosis and treatment are therefore obvious major public health issues.

*C. trachomatis* antibodies have been shown to be a strong indicator of Fallopian tube damage and seem to be superior to hysterosalpingograms as a prognostic indicator of this damage (3, 8-11). The serovars of *C. trachomatis* IgG were found to cause different levels of damage by measuring the ciliar activity in the Fallopian tubes (12) and a number of clinical manifestations (13).

Currently, 18 different serovars of *C. trachomatis* have been identified and have been grouped into three complexes based on antigenic properties: the B-complex (B, Ba, D, E, L1 and L2), the intermediate GF-complex (F, G, K and L3) and the C-complex (A, C, H, I and J) (14, 15). Serovars A, B, Ba and C are primarily found in trachoma, a chronic ocular disease found predominantly in developing countries. Serovars B and Ba have also been found in genital samples. The serovars L1, L2 and L3 cause human lymphogranuloma venereum. The most frequent serovars found in genital infections are D, E and F, and less often serovars G, H, I, J and K (13, 16, 17).

The objective of this study was to examine potential associations between an acute *C. trachomatis* infection (antigens) or a previous *C. trachomatis* infection (antibodies and serovar complexes) with PTB in a population-based cohort. Our second objective was to explore potential associations between *C. trachomatis* complexes and previous fertility problems. The possible association between *C. trachomatis* serovars and PTB as well as fertility problems has not previously been investigated.

### **Patients and Methods**

**Study population.** A population-based prospective cohort study was performed with the purpose of studying genital infection in pregnancy and spontaneous PTB. The participants were pregnant women residing in the geographically defined catchment area

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**Key Words:** *Chlamydia trachomatis*, preterm birth, ectopic pregnancy, infertility, subfertility.

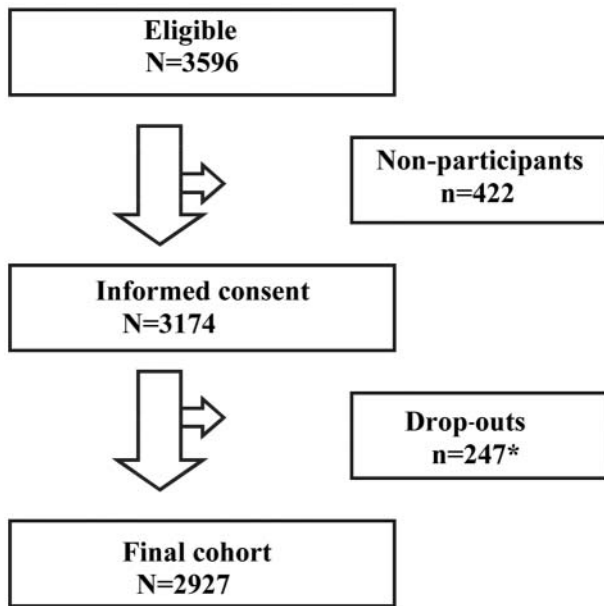


Figure 1. Study population, inclusions and exclusions. \*Other delivery unit ( $n=80$ ), failed to complete questionnaire ( $n=54$ ), moved from catchment area ( $n=35$ ), declined further participation ( $n=32$ ), induced or spontaneous abortion ( $n=22$ ), fetal death ( $n=13$ ), delivered outside hospital ( $n=9$ ), placenta previa (2).

(population about 240,000) of Odense University Hospital, Denmark, who received prenatal care at the hospital from November 1992 to February 1994. Of the 3,596 eligible pregnant women 3,174 (88.4%) agreed to participate in the study and 2,927 (81.4%) completed the study (Figure 1) (18). The study was approved by the Regional Scientific-Ethical Committee of the counties of Vejle and Funen and the Danish Data Protection Agency.

**Sample collection.** All the participating women were enrolled at their first prenatal hospital visit prior to 24 completed weeks of gestation (Mean 17; Range 8-24). At enrolment, a pelvic examination was performed and included a saline wet mount for direct microscopy and the collection of additional samples of venous blood and cervical/vaginal secretion. Maternal blood samples were collected in dry sterile tubes, cooled, centrifuged and aliquoted within 2 h after being drawn. They were then stored at  $-80^{\circ}\text{C}$ . Cervical swabs were examined by ELISA for *C. trachomatis* antigen (19). *C. trachomatis* antibody measurements (IgG and IgM) were performed on serum samples. Determination of *C. trachomatis* IgG and IgM serovars was done using a MicroImmunoFluorescence (MIF) assay described elsewhere (15).

For markers of acute *C. trachomatis* infection, antigen determination was carried out with ELISA and IgM measurements using MIF. However as there were very few women positive for both IgM and the studied outcomes, a positive ELISA for *C. trachomatis* antigen constituted the only definition of acute infection.

**Questionnaires.** Each participant filled out 3 questionnaires, one at enrolment, one at 30 weeks' of gestation, and one after delivery. The questionnaires were all self-administered and covered

questions on previous and current gynaecological/obstetric conditions, medical history. They also included individual indicators of socio-economic status (education and occupation), stress, levels of work-related physical activity, psychosocial factors, smoking, alcohol intake, sexual behaviour during pregnancy including coital frequency. Additionally, the midwife attending the delivery completed a registration form describing the delivery.

**Exposure, confounders and outcome.** The exposure of this study included women with an acute *C. trachomatis* infection in pregnancy and women with IgG indicative of a previous *C. trachomatis* infection, both general and at a serovar level. Acute infection was determined by ELISA on *C. trachomatis* antigen in cervical samples. A previous infection was determined using MIF on *C. trachomatis* IgG in the serum samples. Women with an IgG titre  $\geq 16$  were considered positive. A serovar determination was also conducted in women positive for a previous *C. trachomatis* infection.

The outcome studied was spontaneous PTB, defined as birth prior to 37 completed weeks of gestation after a spontaneous onset of labour. Gestational age was based upon ultrasonographic measurements (biparietal diameter and femur length, week 18) in 97.5% of the participants and in the remaining 2.5% on last menstrual period, only. Furthermore, the opportunity was taken to evaluate any possible retrospective associations between previous *C. trachomatis* infections, both general and at a serovar level, and subfertility (time to pregnancy  $>12$  months), infertility treatment and previous ectopic pregnancy. For this second objective, the exposure was determined after the outcome. In this retrospective part of the study, a possible bias is therefore the uncertainty of the order of infection and outcome. Furthermore, the study population consisted only of women who achieved a pregnancy. This is, therefore, not a population-based study on all women with a previous *C. trachomatis* infection. Hence, the second objective was transformed into a more general hypothesis on serovar complexes and female fertility.

The variables examined for a possible confounding effect were alcohol intake, smoking, age, body mass index, socio-economic status, psychosocial factors, bacterial vaginosis, treatment for chlamydia infection and age at sexual debut. The selection of confounders was based on variations of the crude Odds Ratio (OR) of more than 10%, in accordance with Greenland (20). Relative risks (RR) were used to measure the associations of the cohort, OR were used for all remaining analyses, including logistic regression. All statistical calculations were performed using the SPSS 11.0 statistic software package (SPSS Inc., Chicago, IL, USA).

## Results

The descriptive characteristics of the study population are presented in Table I. The rates of preterm birth (5.8%), spontaneous preterm birth (3.8%), treated for infertility (8.6%), subfertility (17.3%) and ectopic pregnancy (2.7%) were as expected. The MIF test was not carried out for 19 women, one of whom delivered preterm. Eleven percent were positive for unspecified *C. trachomatis* IgG, indicating that they previously had a *C. trachomatis* infection. The cumulative prevalence of the serovars exceeds that of unspecified *C. trachomatis* as some individuals are positive for more than one serovar. Serovar specification failed for 13 out of the 317 positive for general IgG.

Table I. Distribution of basic measures and data on the study population (n=2,927).

	Mean	Range
Age	29 years	18.4-43.6
Body mass index	22.6	14.5-51.2
Gestational age at delivery	279.4 days	184-308
	Number	%
Term birth	2,757	94.2
Preterm birth	170	5.8
Spontaneous preterm birth	112	3.8
Treated for infertility	252	8.6
Subfertile	505	17.3
Previous ectopic pregnancy	78	2.7
Nullipara	1,399	47.8
Twins	81	2.7
Smoking at fertilization	1,156	39.5
Smoking > 10 cig/day	629	21.5
IgG pos at enrollment	317	10.8
IgG B-complex pos	256	8.7
IgG GF-complex pos	75	2.5
IgG C-complex pos	51	1.7
IgG Undefined serovar	13	0.4
Bacterial vaginosis at enrolment	402	13.7

*C. trachomatis* and PTB. Our results show no statistically significant associations between acute *C. trachomatis* infection and spontaneous PTB (Table II). A previous infection, measured by unspecified *C. trachomatis* IgG, however, tended to be associated with spontaneous PTB. The association between the serovar complexes and spontaneous PTB differs between the groups of *C. trachomatis*. Women with the C-complex were at increased risk of spontaneous preterm birth, RR of 2.7 [1.2-6.3], the only serovar complex significantly associated with spontaneous PTB. The B-complex was borderline associated with spontaneous PTB, while no association was found for the GF-complex. Furthermore, none of the theoretical confounders (age, smoking, body mass index, age at sexual debut or bacterial vaginosis) were found to change the crude RR by 10% or more, thus adjusted risk estimates are not presented. For all serovars, the sensitivity and the predictive value of a positive test (PPV) was very low. The aetiological fractions for preterm birth were also very low, varying from 0.2% for the GF-complex, 2.9% for the C-complex and up to 5.5% for the B-complex.

*C. trachomatis* IgG and previous subfertility, infertility treatment and previous ectopic pregnancy. Possible associations between previous *C. trachomatis* infections and fertility and ectopic pregnancy were explored (Table III). Positivity for unspecified *C. trachomatis* IgG was significantly associated with previous subfertility prior to the current pregnancy, with

previous use of infertility treatment to achieve the current pregnancy and with previous ectopic pregnancies prior to this pregnancy. No statistically significant associations were found for the *C. trachomatis* B-complex. However, positivity for the GF-complex was significantly associated with infertility treatment. The strongest associations were found for the C-complex with high ORs for subfertility, infertility treatment and ectopic pregnancy.

*Distribution of C. trachomatis* IgG serovars. Of the women positive for *C. trachomatis* IgG, 256 (80.8%) were B-complex-positive, 75 (23.7%) GF-complex positive and 51 (16.1%) were C-complex positive. Among the 256 positive for serovars of the B-complex, 19 (7.4%) were additionally positive for serovars of the C-complex compared to only 1.2% among the B-complex negative ( $\chi^2=52$ ,  $p<0.001$ ). Likewise, among the B-complex-positive women, 59 (23%) were positive for serovars of the GF-complex compared to only 0.6% among B-complex-negative patients ( $\chi^2=468$ ,  $p<0.001$ ). None were found to be both GF- and C-complex-positive.

To explore whether the adverse outcome was associated with the diverse *C. trachomatis* serovars combinations, ORs or RRs were estimated for both the subgroups of women who were positive for more than one serovar complex, as well as those positive for only one serovar complex (Table IV). No statistically significant associations were found in the analyses of those positive for B-complex and negative for GF-complex, or negative for C-complex (B+, C-, GF-). Positivity for both B- and GF-complex (B+, C-, GF+) was not associated with any of the outcomes, whereas positivity for the GF-complex alone (B-, C-, GF+) was strongly associated with subfertility, infertility treatment and previous ectopic pregnancy. Contrary to the results on the GF-complex, the C-complex shows high ORs both with (B+, C+, GF-) and without the B-complex (B-, C+, GF-). None were positive for both the C- and the GF-complex (B-, C+, GF+ or B+, C+, GF+).

## Discussion

We found no statistically significant association between acute infection with *C. trachomatis* and PTB. However, previous *C. trachomatis* infection (i.e., the presence of unspecified antibodies in serum) tended to be associated with PTB. Furthermore, a strong association was found between the C-complex and PTB, although the number of C-complex positive among women with PTB was very small. The B-complex had a tendency only to be associated with PTB, whereas the GF-complex showed no association with PTB. In the exploratory part of the study, women with C-complex, both with or without the B-complex, and the GF-complex alone (but not in combination with B-complex) were associated with a prior history of subfertility, prior use of infertility treatment and previous ectopic pregnancies.

Table II. *C. trachomatis* and spontaneous PTB.

		Preterm (n=112)	Term (n=2,756)	RR	95% CI	SS	PPV	LR
Acute infection								
<i>C. trachomatis</i> ELISA	Pos	3	62	1.2	[0.4-3.6]	3%	5%	1.2
	Neg	109	2,694					
Previous <i>C. trachomatis</i> infection								
IgG (unspecified)	Pos	17	292	1.5	[0.9-2.5]	15%	6%	1.4
	Neg	94	2,445					
IgG B-complex	Pos	15	234	1.6	[1.0-2.8]	14%	6%	1.6
	Neg	96	2,503					
IgG GF-complex	Pos	3	70	1.1	[0.3-3.2]	3%	4%	1.1
	Neg	108	2,667					
IgG C-complex	Pos	5	44	2.7	[1.2-6.3]	5%	10%	2.8
	Neg	106	2,693					

Relative risks (RR), 95% confidence interval (95% CI), sensitivity (SS), positive predictive value (PPV), likelihood ratio (LR).

Table III. *C. trachomatis* and fertility problems and ectopic pregnancy.

		Subfertile (n=505)			Infertility treatment (n=252)			Previous ectopic pregnancy (n=78)		
		Yes	No	OR [95% CI]	Yes	No	OR [95% CI]	Yes	No	OR [95% CI]
IgG (unspecified)	Pos	86	231	1.9 [1.4-2.5]	53	259	2.4 [1.7-3.4]	16	301	2.2 [1.2-3.8]
	Neg	417	2,173		199	2,359		62	2,528	
IgG B-complex	Pos	55	201	1.3 [0.9-1.8]	25	227	1.2 [0.8-1.8]	7	249	1.0 [0.7-2.2]
	Neg	448	2,203		227	2,391		71	2,580	
IgG GF-complex	Pos	17	58	1.4 [0.8-2.4]	12	60	2.1 [1.1-4.0]	4	71	2.1 [0.7-6.0]
	Neg	486	2,346		240	2,558		74	2,758	
IgG C-complex	Pos	24	27	4.4 [2.5-7.7]	20	31	7.2 [4.0-12.8]	6	45	5.2 [2.2-12.4]
	Neg	479	2,377		232	2,587		72	2,784	

Odds ratio (OR), 95% confidence interval (95% CI).

**Acute *C. trachomatis* infection and PTB.** Women with acute *C. trachomatis* infections during pregnancy have a 2-3 fold increased risk of spontaneous PTB (21). This finding is supported by other studies examining associations between *C. trachomatis* infections and adverse pregnancy outcome (7). We were not able to consolidate this finding in our low-risk population where the prevalence of an acute *C. trachomatis* infection was only 2.3% overall and 2.7% among women delivering preterm. The study by Andrews *et al.* was a nested case-control study consisting of 190 cases of preterm birth and 190 matched controls, all recruited prior to 24 weeks of gestation. In this American study, the overall prevalence of acute *C. trachomatis* infection was 11% and the prevalence among cases was 15.8% (21). Since their study was a nested case-control study, the overall prevalence in the two studies is not directly comparable. The prevalence among women delivering preterm however,

demonstrates a substantial difference between these two populations. This disparity may explain the diverging conclusions of these studies.

**Previous *C. trachomatis* infection and PTB.** Our study focuses, not only on the impact of previous infections but also on the impact of the serovar-complex. Only few studies have addressed the plausible impact of *C. trachomatis* serovars, while most have examined the distribution of serovars in different populations and by a range of clinical symptoms (5, 13, 16, 17, 22, 23). The results of some studies indicated the serovars in the C-complex to be the most destructive (12, 13). Our findings support this as we found that *C. trachomatis* C-complex serovars had an increased risk for PTB. Previous *C. trachomatis* infections obviously explain only a small fraction of spontaneous PTBs, which is in line with the current presumption of a multifactorial aetiology of spontaneous PTB.

Table IV. Serovar complex combination or separation and fertility problems and obstetric outcome.

	Subfertile			Infertility treatment			Previous ectopic pregnancy			Spontaneous preterm birth		
	Yes	No	OR [95% CI]	Yes	No	OR [95% CI]	Yes	No	OR [95% CI]	Yes	No	RR [95% CI]
B-, C-, GF-	353	2,250	1.0	208	2,363	1.0	64	2,539	1.0	94	2,509	1.0
B-, C-, GF+	8	8	6.4[2.4-17.1]	8	7	13.0[4.7-36.2]	3	13	9.2[2.5-32.9]	0	16	-
B-, C+, GF-	13	19	4.4[2.1-8.9]	11	21	5.9[2.8-12.5]	4	28	5.7[1.9-16.6]	2	29	1.8[0.5-6.8]
B-, C+, GF+	0	0	-	0	0	-	0	0	-	0	0	-
B+, C+, GF-	10	9	7.1[2.9-17.6]	9	10	10.2[4.1-25.4]	2	17	4.7[1.1-20.6]	3	15	4.5[1.6-13.0]
B+, C+, GF+	0	0	-	0	0	-	0	0	-	0	0	-
B+, C-, GF+	7	52	0.9[0.4-1.9]	4	53	0.9[0.3-2.4]	1	58	0.7[0.1-5.0]	3	54	1.4[0.5-4.4]
B+, C-, GF-	27	151	1.1[0.7-.7]	12	164	0.8[0.5-1.5]	4	174	0.9[0.3-2.5]	9	165	0.8[0.2-3.5]

Odds ratio (OR), 95% confidence interval (95% CI), relative risks (RR).

The examination of *C. trachomatis* and fertility outcomes was more exploratory in nature, since the exposure (previous *C. trachomatis* infection) was determined after the outcome (fertility problems prior to the current pregnancy). In addition, the study population consisted only of women who became pregnant, and excluded infertile women. Based on the assumption that most of the infections occurred prior to the outcome, our results show that the women positive for serovars of the C-complex were at a higher risk of being subfertile, of having been treated for infertility and of a previous ectopic pregnancy than those positive for serovars of the B- and GF-complex (Table III).

The current association found in this study between *C. trachomatis* (unspecified antibodies) and previous ectopic pregnancy is in accordance with that of other studies (24-26). Although the numbers are small, we found a high association between C-complex-positive women and previous ectopic pregnancy [OR 5.2 (2.2-12.4)] (Table III). Like many other studies, this study enrolled pregnant women as both cases and controls, and excluded women who did not become pregnant. The reason for this might be damage caused by a *C. trachomatis* infection, including the C-complex. Leng *et al.* (12) showed that the C-complex led to total hindrance of the ciliary activity in the Fallopian tubes, causing an increased risk of ectopic pregnancy. Therefore it could be assumed that the proportion of women who were positive for *C. trachomatis*, and in particular serovars of the C-complex, is greater among sterile women. Exclusion of these women could thus underestimate the true association between having had a *C. trachomatis* infection and the fertility outcomes studied.

The exploration of the effect of the combination of serovar complexes confirmed the C-complex to be the most destructive. Unfortunately, our numbers are too insignificant to lead to further conclusions. The impact of the C-complex found in our study is in line with previous findings. One group reported a higher rate of complicating

salpingitis among C-complex positive women (5). Other authors found that C-complex positive women bled more often when cervical swabs were performed and more frequently had elevated leukocyte counts (13). However, Persson and Osseir (27) found various clinical manifestations to occur at similar rates in the different serovar-complexes. A Dutch study found that C-complex-positive women had a history of pelvic inflammatory disease less often (17).

The strength of this study is that it is a large population-based study with a low drop-out rate. Additionally, all the women enrolled were examined for *C. trachomatis* antigens, antibodies and serovar complexes. Furthermore, due to the detailed questionnaires, this study had access to a wide range of information, providing the possibility to examine several possible confounders. However, none of the previously published confounders in related studies were found here to significantly change the risk estimates.

In spite of the size of the cohort, a weakness of our study is the low number of pregnant women who were positive for the three serovar complexes. Although this number is in accordance with the distribution found in other studies, larger numbers would generate more accurate risk estimates expressed by smaller confidence intervals. It would also have been desirable to have detailed information on the single serovar level and not just on the level of the serovar complexes.

Regarding the aspect of fertility problems and previous ectopic pregnancies, one major limitation of this study is the exclusion of women unable to conceive. Selection bias might also be present due to the fact that these three outcomes occurred prior to our record of exposure. These limitations do, however, not apply to the associations between the serovar complexes and PTB.

We are not aware of previous studies on *C. trachomatis* complexes and fertility problems or PTB. We found a previous *C. trachomatis* C-complex infection to be associated with PTB, and potentially also with ectopic

pregnancy, subfertility and infertility. This information provides a basis for further research on *C. trachomatis* serovars and adverse outcome. To be able to draw definitive conclusions on associations between *C. trachomatis* C-complex and the risk of subfertility, infertility treatment and ectopic pregnancy, studies where the *C. trachomatis* status is examined prior to the outcome are needed.

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## References

- Gerbase AC, Rowley JT and Mertens TE: Global epidemiology of sexually transmitted diseases. *Lancet* 351 Suppl 3: 2-4, 1998.
- Land JA and Evers JL: Chlamydia infection and subfertility. *Best Pract Res Clin Obstet Gynaecol* 16: 901-912, 2002.
- Rhoton-Vlasak A: Infections and infertility. *Prim Care Update Ob Gyns* 7: 200-206, 2000.
- Mardh PA: Influence of infection with *Chlamydia trachomatis* on pregnancy outcome, infant health and life-long sequelae in infected offspring. *Best Pract Res Clin Obstet Gynaecol* 16: 847-864, 2002.
- Persson K and Osser S: Serovars of *Chlamydia trachomatis* causing postabortion salpingitis. *Eur J Clin Microbiol Infect Dis* 8: 795-798, 1989.
- Eggert-Kruse W, Rohr G, Demirakca T *et al*: Chlamydial serology in 1303 asymptomatic subfertile couples. *Hum Reprod* 12: 1464-1475, 1997.
- Paavonen J and Eggert-Kruse W: *Chlamydia trachomatis*: impact on human reproduction. *Hum Reprod Update* 5: 433-447, 1999.
- Dabekaussen YA, Evers JL, Land JA *et al*: *Chlamydia trachomatis* antibody testing is more accurate than hysterosalpingography in predicting tubal factor infertility. *Fertil Steril* 61: 833-837, 1994.
- Mol BW, Dijkman B, Wertheim P *et al*: The accuracy of serum chlamydial antibodies in the diagnosis of tubal pathology: a meta-analysis. *Fertil Steril* 67: 1031-1037, 1997.
- Veenemans LM and van der Linden PJ: The value of *Chlamydia trachomatis* antibody testing in predicting tubal factor infertility. *Hum Reprod* 17: 695-698, 2002.
- Thomas K, Coughlin L, Mannion PT *et al*: The value of *Chlamydia trachomatis* antibody testing as part of routine infertility investigations. *Hum Reprod* 15: 1079-1082, 2000.
- Leng Z, Moore DE, Mueller BA *et al*: Characterization of ciliary activity in distal Fallopian tube biopsies of women with obstructive tubal infertility. *Hum Reprod* 13: 3121-3127, 1998.
- van Duynhoven YT, Ossewaarde JM, Derksen-Nawrocki RP *et al*: *Chlamydia trachomatis* genotypes: correlation with clinical manifestations of infection and patients' characteristics. *Clin Infect Dis* 26: 314-322, 1998.
- Treharne JD: *Chlamydia trachomatis*: serological diagnosis. *Infection* 10 Suppl 1: S25-S31, 1982.
- Wang SP, Grayston JT, Alexander ER *et al*: Simplified micro-immunofluorescence test with trachoma-lymphogranuloma venereum (*Chlamydia trachomatis*) antigens for use as a screening test for antibody. *J Clin Microbiol* 1: 250-255, 1975.
- Kuo CC, Wang SP, Holmes KK *et al*: Immunotypes of *Chlamydia trachomatis* isolates in Seattle, Washington. *Infect Immun* 41: 865-868, 1983.
- van de Laar MJ, van Duynhoven YT, Fennema JS *et al*: Differences in clinical manifestations of genital chlamydial infections related to serovars. *Genitourin Med* 72: 261-265, 1996.
- Thorsen P, Schendel DE, Deshpande AD *et al*: Identification of biological/biochemical marker(s) for preterm delivery. *Paediatr Perinat Epidemiol* 15 Suppl 2: 90-103, 2001.
- Thorsen P, Jensen IP, Jeune B *et al*: Few microorganisms associated with bacterial vaginosis may constitute the pathologic core: a population-based microbiologic study among 3596 pregnant women. *Am J Obstet Gynecol* 178: 580-587, 1998.
- Greenland S: The effect of misclassification in the presence of covariates. *Am J Epidemiol* 112: 564-569, 1980.
- Andrews WW, Goldenberg RL, Mercer B *et al*: The Preterm Prediction Study: association of second-trimester genitourinary chlamydia infection with subsequent spontaneous preterm birth. *Am J Obstet Gynecol* 183: 662-668, 2000.
- Moncan T, Eb F and Orfila J: Monoclonal antibodies in serovar determination of 53 *Chlamydia trachomatis* isolates from Amiens, France. *Res Microbiol* 141: 695-701, 1990.
- Workowski KA, Suchland RJ, Pettinger MB *et al*: Association of genital infection with specific *Chlamydia trachomatis* serovars and race. *J Infect Dis* 166: 1445-1449, 1992.
- Sheffield PA, Moore DE, Voigt LF *et al*: The association between *Chlamydia trachomatis* serology and pelvic damage in women with tubal ectopic gestations. *Fertil Steril* 60: 970-975, 1993.
- Osser S, Persson K and Liedholm P: Tubal infertility and silent chlamydial salpingitis. *Hum Reprod* 4: 280-284, 1989.
- Sherman KJ, Daling JR, Stergachis A *et al*: Sexually transmitted diseases and tubal pregnancy. *Sex Transm Dis* 17: 115-121, 1990.
- Persson K and Osser S: Lack of evidence of a relationship between genital symptoms, cervicitis and salpingitis and different serovars of *Chlamydia trachomatis*. *Eur J Clin Microbiol Infect Dis* 12: 195-199, 1993.

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