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Association between *Chlamydia trachomatis* antibodies and subfertility in the Northern Finland Birth Cohort 1966 (NFBC 1966), at the age of 31 years

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SUMMARY

The objective of this study was to assess the serological association between previous *Chlamydia trachomatis* infection and subfertility in a general population sample. A nested case ($n=493$)-control ($n=986$) study in a population-based birth cohort consisting of 12 058 live births from the year 1966 was conducted. The analysis was restricted to those 6007 cohort members who replied to a postal inquiry and participated in a health examination including blood samples at the age of 31 years. The presence of *C. trachomatis*-specific serum IgG antibodies was screened by a synthetic peptide-based enzyme-linked immunosorbent assay. All the positive sera were further tested by the microimmunofluorescence method using immunotype pools and individual immunotypes of *C. trachomatis* as antigens. An association was found between the detection of immunotype-specific *C. trachomatis* antibodies and subfertility both in men and women. The results of the present study confirm the serological association between past *C. trachomatis* infections and subfertility in male or female partners of the couple in the population-based sample.

INTRODUCTION

Chlamydia trachomatis is the most common cause of sexually transmitted bacterial infections throughout the world, and the morbidity associated with chlamydial infections is high. Approximately 3% of women with chlamydial lower genital tract infection ultimately develop infertility, but in males, evidence of the link between *C. trachomatis* and infertility is more limited [1], and there is a controversy concerning the role of both symptomatic and asymptomatic *C. trachomatis* infections in the aetiology of male infertility [2–7].

In earlier studies, the study populations have mainly consisted of clients of infertility clinics. As far as we know, there are no general population-based studies on the association between the detection of serum *C. trachomatis* antibodies and infertility or subfertility. The time to pregnancy in months (TTP; the time elapsed from the date the couple started trying to conceive, to the time the woman becomes pregnant) approach is the best-known measure of infertility at the population level, and it is widely used as a definition of subfertility [8, 9]. TTP has not been applied in this context earlier.

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Table 1. *Answers to the questions used to define time to pregnancy (TTP) in the Northern Finland Birth Cohort 1966 (n=8690*) at the age of 31 years*

Question	Female	Male	All (%)
Did you use any contraception during the conception of the first pregnancy?			
(1) No pregnancies	997	1404	2401 (30)
(2) Yes, only occasionally	204	89	293 (4)
(3) Yes, almost all the time	147	72	219 (3)
(4) Yes, all the time	144	49	193 (2)
(5) No	2850	2072	4922 (61)
Total (*n*)	4342	3686	8028† (100)
If you chose the answer number (1) and you are currently trying to get pregnant, indicate how long you have been trying ____ months:			
TTP <12 months	97	152	249 (60)
TTP ⩾12 months	90	75	165 (40)
Total (*n*)	187	227	414 (100)
If you chose the answer number (5): How many months did it take to get pregnant or to conceive? ____ months			
TTP <12 months	2126	1446	3572 (86)
TTP ⩾12 months	366	220	586 (14)
Total (*n*)	2492	1666	4158 (100)

* All those who responded to the postal questionnaire.
† 662 (8%) missing.

The purpose of this study was to investigate the association between antibodies to *C. trachomatis* and subfertility, measured as TTP in a large population-based sample of males and females from the Northern Finland Birth Cohort (NFBC 1966) at the age of 31 years.

METHODS

Birth cohort and data collection

The original study population was comprised of 12 231 persons from the two northernmost provinces of Finland, Oulu and Lapland who were born in 1966, covering 96% of all eligible births in this region (NFBC 1966) [10]. The cohort has been prospectively followed since the prenatal period. During 1997–1998, questionnaires were sent to all 11 637 who were still alive (75% response, $n=8690$). Those still living in the original area or who had moved to the capital area were invited for clinical examinations, and 3127 women and 2880 men attended, gave a blood sample and written consent ($n=6007$, 70% of those eligible). The subjects were representative of the whole cohort in terms of their social background in early childhood. The present study is based on 4158 subjects for whom data on TTP of the first pregnancy was available and 414 subjects who had currently been trying to conceive having no earlier pregnancies (Table 1).

Inclusion and exclusion criteria for the nested case-control study

We conducted a nested case-control study, in which TTP of 12 months or more before the first pregnancy was defined as subfertility. The factors used to determine TTP are presented in Table 1 [9]. Those who had used contraception at the time of conception were excluded ($n=705$). There were 751 subjects with TTP ⩾12 months, and 493 (304 females) of them gave blood samples. Two randomized controls for each case ($n=986$) were enrolled from those who had had TTP <12 months before the conception of their first pregnancy, had not used contraception at the time of conception, had not felt infertility to be a problem, and had not been examined or treated because of infertility. Eight cases (three females) and their control subjects had to be excluded due to lost blood samples. Additionally, six cases (three females, three males) and three controls (one female, two males) were excluded due to inconsistent answers to the key questions. Thus, we had in the final analysis 479 cases

Table 2. *Questions used for case selection* (*reported subfertility*)

1. Has infertility ever been a problem for you or is it a problem right now?
   (1) Yes (earlier or at this moment)
   (2) No
   (3) I do not know, because I have not tried to get pregnant or conceive
2. Have you or your partner been examined for infertility?
   (1) No
   (2) Yes (self, partner or both)
3. Has a reason for infertility been found in
   (1) Yourself
   (2) Your partner
   (3) Both
   (4) No reason has been found
4. Have you been treated for infertility? (You can choose more than one alternative)
   (1) No
   (2) Yes, hormonal treatment
   (3) Yes, operative treatment
   (4) Yes, *in vitro* fertilization or insemination
   (5) Other, which

(298 female and 181 male partners of subfertile couples) and 967 controls (600 female and 367 male partners of fertile couples). A pre-study calculation indicated that when assuming 20% occurrence of *C. trachomatis* antibodies in the general female population this study has 90% power to find a 10% difference in antibody occurrence between the female cases and the controls at 5% significance level. When assuming a 10% occurrence of antibodies at the general male population a 10% difference between male cases and controls would be detectable with an average 80% power at 5% significance level [5, 11, 12]. The study protocol was approved by the Ethics Committee of the University of Oulu.

Variables and measurements

The explanatory variables were the presence of *C. trachomatis* antibodies and a self-reported, doctor-diagnosed and confirmed history of *C. trachomatis* infection or pelvic inflammatory disease (PID). The main outcome variable for case selection was reported subfertility which was investigated, in addition to TTP, with questions shown in Table 2.

The postal questionnaire included the following questions about genital infections diagnosed by a general practitioner or consultant: 'Have you ever had genital chlamydial infection? [no/yes]' and 'Have you ever had salpingo-oophoritis? [no/yes]'. In Finland diagnostic laboratory tests have always been routinely performed to confirm the diagnosis of *C. trachomatis* infection. The number of the subject's own children was requested.

Laboratory examinations

Serum samples were stored at $-20\ ^{\circ}C$ until analysed in 2000. *C. trachomatis*-specific IgG antibodies were first screened by a synthetic peptide-based EIA test (Labsystems, Helsinki, Finland). We used lower cut-off values (1·0) for positivity than those recommended by the manufacturer in order to also include cases with lower antibody levels. All the cases above the 1·0 cut-off were further tested by microimmunofluorescence (MIF) [13] using elementary bodies of *C. trachomatis*-pooled serovars G, F and K (intermediate complex), B, E and D (B complex) and C, J, H and I (C complex) as antigens. *C. pneumoniae* antibodies as a control were measured using the Finnish strain Kajaani 6 as the antigen. The serotype-specific antigens C, J, H, I, F and K were obtained from the Washington Research Foundation (Seattle, WA, USA) and G, B, E and D from ATCC (American Type Culture Collection, Rockville, MD, USA). All the serotype-specific antigens were tested and diluted before pooling. Reactivity of the antigen pools was checked by using a commercial *C. trachomatis* monoclonal antibody (Bio-Rad, Redmond, WA, USA), which identifies 15 serological types. The specificity of the antigen pools was tested with known positive sera from previous studies. The positive control sera with known antibody titres were included in every test series and they gave the same titre in all titrations. One person prepared antigen slides with the same antigen densities and quality for all experiments. The interpretation of the results was done by the same experienced reader (M.P.) and by using the same microscope.

If there were any antibodies against the serovar pool present, all the *C. trachomatis* immunotypes of the pool were further analysed. Fluorescein-conjugated anti-human IgG (Kallestadt Diagnostic, Chaska, MN, USA) was used as a conjugate. Sera were tested to the end-point, starting at 1 in 8 dilutions.

Statistical analyses

Antibody titres were transformed to natural logarithms for the calculation of their geometric means

Table 3. *Frequencies and geometric mean titres of* C. trachomatis *antibody against serovar pools measured by MIF in cases* ($n=479$) *and controls* ($n=967$)

	Cases			P†	
	TTP ⩾12 months	TTP ⩾24 months*	Controls	TTP ⩾12	TTP ⩾24
Men	$n=181$	$n=90$	$n=367$		
MIF					
IgG positive, % (n)	7·7 (14)	10·0 (9)	5·7 (21)	0·365	0·142
GMT (95% CI)	4·6 (4·3–4·9)	4·7 (4·2–5·3)	4·3 (4·2–4·5)	0·330	0·126
Women	$n=298$	$n=133$	$n=600$		
MIF					
IgG positive, % (n)	9·7 (29)	8·3 (11)	9·2 (55)	0·784	0·744
GMT (95% CI)	4·9 (4·5–5·3)	4·7 (4·2–5·2)	4·6 (4·4–4·8)	0·702	0·790

GMT, Geometric mean titre; CI, confidence interval.
* Subgroup of cases.
† χ^2 test for categorical variables and Mann–Whitney U test for continuous variable.

(GMT) and 95% confidence intervals (CI). The Mann–Whitney U test was used to compare continuous variables between the study groups due to the originally skewed distributions. In the case of categorical variables, the groups were compared with the χ^2 test or Fisher's exact test when appropriate. Spearman's correlation coefficient was used for an analysis of correlations. We used the SPSS software for Windows (version 9.0, SPSS Inc., Chicago, IL, USA).

RESULTS

In our original study population ($n=8690$), 8% of the males and 10·5% of the females respectively, reported subfertility (TTP ⩾12 months) in the couple's relationship. Among 479 cases, 51% ($n=93$) of men and 53% ($n=159$) of women reported that either themselves, their partner or both of the partners had been examined for infertility. Of these 252 cases, 48% (75 females and 47 males) reported that no reason was found. By interview at age 31 years, 75% of the male cases and 74% of the female cases had finally had children of their own. There were no clear differences in the incidences of self-reported genital infections between the cases and the controls, except the difference (not reaching statistical significance, $P=0·065$) in the incidence of PID between female cases (7·0%) and their controls (4·2%).

In the EIA screening, 14·4% of male cases and 9·3% male controls had *C. trachomatis* IgG antibodies in serum and corresponding percentages for female cases and controls were 16·8 and 14·8% respectively. The frequencies and geometric mean titres of MIF antibodies to *C. trachomatis* serovar pools (all pools combined) in the cases and controls are shown in Table 3. Although cases had IgG antibodies present more often and in higher titres, no statistically significant difference was found.

The analysis of serovar pool-specific *C. trachomatis* IgG antibodies (Table 4) showed that the most clear differences between cases and controls were found in antibodies against C complex containing immunotypes C, J, H and I: 7·7% of the sera of the male cases and 3·0% of the sera of their controls showed IgG antibody specificity for pooled C, J, H and I serotypes ($P=0·012$), and the corresponding figures among the women were 8·1% and 5·5% ($P=0·139$). Among the single *C. trachomatis* serotypes the frequency of IgG antibodies was generally higher for the cases than the controls; significantly to serotype H among males and to serotypes C, J, H and I among females (Table 4).

Table 5 shows that *C. trachomatis* antibodies were more often present in males with self-reported prior chlamydial diagnosis compared to those with no self-report of *C. trachomatis* infection (cases: 17·6 and 6·7%; controls: 12·2 and 4·9% respectively). The same phenomenon was also seen in females (cases: 21·1 and 8·1%; controls: 23·5 and 7·3%). No statistically significant difference in the presence of antibodies was demonstrated between cases and controls.

The prevalence of IgG antibodies to *C. pneumoniae* was similar in all male cases (61%) and their controls (61%) as well as in female cases (46%) and their

Table 4. *Frequencies of IgG antibodies to different* C. trachomatis *pools and individual serotypes in cases and controls*

	Men			Women		
Serotype	Cases ($n=181$) % (n)	Controls ($n=367$) % (n)	P*	Cases ($n=298$) % (n)	Controls ($n=600$) % (n)	P*
B, E, D	6·1 (11)	5·2 (19)	0·663	8·4 (25)	8·8 (53)	0·824
G, F, K	2·2 (4)	0·5 (2)	0·096	5·7 (17)	5·2 (31)	0·736
C, J, H, I	7·7 (14)	3·0 (11)	0·012	8·1 (24)	5·5 (33)	0·139
B	3·3 (6)	2·5 (9)	0·584	7·7 (23)	6·8 (41)	0·627
E	3·9 (7)	1·9 (7)	0·247	4·4 (13)	5·7 (34)	0·409
D	2·8 (5)	2·2 (8)	0·767	5·4 (16)	4·7 (28)	0·646
G	1·1 (2)	0·3 (1)	0·255	3·0 (9)	2·3 (14)	0·540
F	0·6 (1)	0·0 (0)	0·330	3·0 (9)	2·5 (15)	0·649
K	1·7 (3)	0·3 (1)	0·108	3·4 (10)	4·0 (24)	0·634
C	2·8 (5)	1·9 (7)	0·543	7·0 (21)	3·8 (23)	0·036
J	3·9 (7)	1·9 (7)	0·247	8·1 (24)	4·3 (26)	0·022
H	4·4 (8)	1·4 (5)	0·036	6·7 (20)	3·3 (20)	0·021
I	3·9 (7)	1·6 (6)	0·135	7·0 (21)	3·7 (22)	0·025
Any serotype	6·6 (12)	4·9 (18)	0·404	9·4 (28)	9·0 (54)	0·846
$\geqslant$2 serotypes	5·5 (10)	2·5 (9)	0·064	8·7 (26)	7·0 (42)	0·358

* χ^2 test or Fisher's exact test.

Table 5. *Frequencies of EIA and MIF antibodies in cases and controls with and without a self-reported prior diagnosis of* Chlamydia trachomatis *infection verified by a medical practitioner or consultant*

		History of verified *C. trachomatis* infection					
		Yes			No		
		Cases % (n)	Controls % (n)	P*	Cases % (n)	Controls % (n)	P*
Men		$n=17$	$n=41$		$n=164$	$n=326$	
EIA	IgG $\geqslant$1·0	23·5 (4)	17·1(7)	0·715	13·4 (22)	8·3 (27)	0·074
MIF	IgG positive	17·6 (3)	12·2 (5)	0·681	6·7 (11)	4·9 (16)	0·410
Women		$n=38$	$n=68$		$n=259$	$n=532$	
EIA	IgG $\geqslant$1·0	28·9 (11)	32·4 (22)	0·717	15·1 (39)	12·6 (67)	0·340
MIF	IgG positive	21·1 (8)	23·5 (16)	0·770	8·1 (21)	7·3 (39)	0·698

* χ^2 test or Fisher's exact test.

controls (49%). No correlation between *C. pneumoniae* IgG antibodies measured by MIF and *C. trachomatis* IgG antibodies measured by EIA or by MIF (Spearman's correlation coefficient: $r_s=0{\cdot}001$, $r_s=0{\cdot}013$) was found in the whole study population ($n=1446$).

DISCUSSION

Our aim was to assess the association between *C. trachomatis* antibodies and subfertility in the population-based nested case-control study including both genders at the age of 31 years. The prevalence of subfertility measured with TTP is in accordance with earlier studies, being approximately 10% [14, 15]. Interestingly, in the male partners of subfertile couples, *C. trachomatis* antibodies were even more clearly associated with subfertility than in women. The role of *C. trachomatis* infection in male infertility has remained controversial, but the present study suggests that it is also important in males. Our findings point to the possibility that untreated and chronic *C. trachomatis* infections may lead to elevated antibody levels, also in male cases.

Chlamydial serology is a controversial topic. Wang and Grayston [13] established the prototype MIF test based on the use of individual spots of formalinized elementary bodies of different chlamydial serotypes next to each other. EIA tests have been developed to replace the time-consuming and complex MIF, which is kept as the gold standard, but even the best tests, e.g. peptide-based EIAs, do not provide peptides to all the known 18 serotypes of *C. trachomatis*. Due to the technical difficulties associated with massive screening by MIF test, we used peptide-EIA as the screening method for *C. trachomatis* antibodies. Only the sera positive by peptide-EIA were further tested by MIF, using three pools of different serotypes as antigens, and if the pool gave a positive reaction, individual serotypes were tested. We used commercial peptide-EIA, utilizing synthetic peptides from the variable major outer membrane protein (MOMP) domain IV of *C. trachomatis* serotypes C, G, E and L2, and this method has been shown to be very sensitive in the measurement of *C. trachomatis* antibodies [16]. Furthermore, we used lower cut-off values for positivity than those recommended by the manufacturer in order to detect lower antibody levels. Thus, it is unlikely that by using the peptide-EIA test for screening, we missed any antibody-positive cases. It has also been claimed that false-positive *C. trachomatis* antibody results may occur due to cross-reactivity with *C. pneumoniae* antibodies [17, 18]. In the present study no correlation was found between *C. trachomatis* and *C. pneumoniae* antibodies.

Cumulative incidence of diagnosed *C. trachomatis* infections in our study in a general population of 31-year-old subjects was 11–12% (Table 2). To our knowledge there are no cumulative incidence data on *C. trachomatis* infections in earlier studies. In a recent study [19] the prevalence of chlamydia in Finland was 8·4% in the sexually transmitted disease clinics and 5·3% in general clinics. The prevalence was highest in the youngest age group (15–19 years; 16% in females and 14% in males). In the next two 5-year age groups, the prevalence was clearly lower in women but remained high in men [19].

In males, antibodies against the C complex and serotype H were significantly more common in the cases than the controls. These findings suggest that serotype H might be more strongly associated to be the development of male infertility than other *C. trachomatis* serotypes. Transmission of undiagnosed infection from the male to female partner in couples may cause subfertility through tubal damage. We do not know the consequences of untreated infection on semen quality. In the literature, the association between *C. trachomatis* antibodies and male infertility is limited and the impact of chlamydial infection on semen quality is controversial [1]. It has been suggested that serum antibodies may not be reliable markers of the previous or current exposure to *C. trachomatis* infection [20]. However, the presence of chlamydial IgG or IgA antibodies measured by insensitive single-antigen MIF in seminal plasma, suggesting a previous sexually transmitted infection, has been shown to be related to chlamydial IgG antibodies in serum [21]. Eggert-Kruse et al. [22] did not find any relationship between chlamydial IgG antibodies in serum and semen quality in subfertile men. However, chlamydial antibodies in semen were related to chlamydial IgG antibodies in serum of the female partners obtained at the same time. These findings suggest that the main influence of *C. trachomatis* on male fertility is based on sexual transmission and a negative effect on the tubal function of the female partner, but not on reduced functional capacity of sperm [21, 22].

In females, a clear difference in specific antibodies to C, J, H and I serotypes of *C. trachomatis* measured was demonstrated between cases and controls. In women, the association between *C. trachomatis* antibodies and tubal subfertility has been known since 1979 [23]. Superficial infections, e.g. cervicitis, are considered to provide a poor stimulus for antibody production, whereas infiltrating genital infections are associated with seroconversion [24].

In our study, the differences in *C. trachomatis* antibodies between the cases and controls could be attributed to the C complex and, inside it, to the single serotypes C, J, H and I in females and serotype H in males. The following distribution of serotypes in Finland in a decreasing order of prevalence has been reported: B, E and D, G and F, C and J, K, H, and I, the prevalence of the C complex being approximately 20% of the isolates and serological findings [25]. There is no powerful evidence to suggest that specific genital syndromes or clinical manifestations, such as PID, are specifically linked to certain serotypes [1]. However, Dean et al. [26] have shown that almost all patients with recurrent *C. trachomatis* infection are infected with uncommon serotypes of the C complex, suggesting that the C complex is associated with chronic or recurrent infections.

Due to the study design, the blood sampling was performed at the age of 31 years and not during the

time of conception of the first pregnancy. However, the age of peak prevalence for *C. trachomatis* infection has been shown to be 15–24 years [27, 28]. Furthermore, if the blood sampling had been performed at an earlier age the levels of antibodies to *C. trachomatis* would probably have been higher but then the information about subfertility would have been scarce. In our cohort population, the mean age at the first birth was 25·5 years (M.-R. Järvelin, unpublished results), which means that most of the persons with *C. trachomatis* infection who belonged to the present study population had had the infection before their first pregnancy.

Chlamydial infections cause major medical, social and economic problems. Although according to the different studies tuboperitoneal factors are the main cause of subfertility in only 14–30% of cases [14, 15], tubal factor subfertility is the most important preventable cause of subfertility. Since asymptomatic infection is common in both men and women, screening of sexually active adolescents for chlamydial infections has been proposed [29–32]. Our findings support at least indirectly the suggestion that the screening (using PCR/LCR) of young men might be useful in preventing the long-term consequence of untreated infections [33, 34].

This unique study population provided us with the possibility to evaluate the role of *C. trachomatis* infection in the general population and in the early phase of infertility problems and not in the biased population seeking help from infertility clinics. Our results confirm the serological association between *C. trachomatis* infections and subfertility and the rather high incidence of undiagnosed *C. trachomatis* infections in male partners of subfertile couples. Thus, the results of the present study further suggest that serology might be a useful screening method in infertility investigations.

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