The Relationship of *Chlamydia trachomatis* Infection and Male Infertility

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ABSTRACT

Objectives. Infertility affects at least 2 million couples in the United States. One third of infertility is attributed to male causes, but the etiology of most male infertility remains obscure. This study was designed to investigate the relationship between *Chlamydia trachomatis* and unexplained infertility in men.

Methods. Questionnaires and serum were collected prospectively from 52 case subjects (men from couples with explicitly defined idiopathic infertility) and 79 control subjects (first-time expectant fathers).

Results. Case subjects were significantly more likely than control subjects to be seropositive for antibody to *C trachomatis* at a titer of 1:64 or higher. By test of trend, higher titers were associated with higher odds ratios. Adjustment for age of either partner at initiation of pregnancy attempt, race, income, previous genitourinary symptoms or diagnoses, number of previous sexual partners, and barrier contraceptive use had no significant effect on the estimate of the odds ratio. One half of the men who were antibody positive had no history of genitourinary symptoms.

Conclusions. Our results suggest an association between infection with *C trachomatis* in men and unexplained infertility and imply that infection is frequently asymptomatic. (*Am J Public Health.* 1993;83:996–1001)

Introduction

Infertility, defined as the inability to conceive after 1 year of unprotected intercourse, occurs in 15% of US couples. A conservative estimate of the number of couples desiring but unable to achieve pregnancy is 2 million.[1,2] Approximately one third of infertility is thought to be due to male factors, one third to female factors, and one third to a combination of the two.[3]

Male infertility factors are broadly categorized as primary testicular failure (sperm dysfunction), secondary testicular failure (endocrine dysfunction), and post-testicular obstruction.[4] Some of the underlying causes are straightforward, such as azoospermia resulting from chemotherapy. Infectious causes with known pathophysiologies include postgonococcal ductal obstruction and mumps-related orchitis with testicular hypofunction. Exact etiologies of male infertility are rarely determined, however, and most men's infertility is attributed to sperm dysfunction of unknown etiology.[5]

Although there is considerable speculation that male-factor infertility may be related to other infectious agents, such as ureaplasma and chlamydia, studies have remained inconclusive.[6–8] The demonstration of a relationship between *Chlamydia trachomatis* and male infertility would have important public health consequences. The Centers for Disease Control estimated that in 1981 approximately 2.1 million cases of nongonococcal urethritis occurred; between 25% and 70% of these illnesses were attributable to chlamydia alone.[9] Therefore, we designed a case-control study to address the following main question: Is male infection with *C trachomatis,* measured by the presence of serum antichlamydial antibody, associated with male infertility? The relationship of antichlamydial antibody to semen parameters was also examined.

Methods

Case Subject Definition

Case subjects were defined as male partners of couples enrolled at one hospital-based infertility practice and one health maintenance organization-based infertility practice, which was affiliated with the study hospital. The demographic composition of the hospital's ambulatory patient population was as follows: White, 71%; Black, 15%; other, 14%. Eighty-two percent of the ambulatory patients were covered by private medical insurance.

Men were enrolled as case subjects if they met the following criteria: (1) the couple had undertaken at least 1 year of unprotected intercourse without conceiving; (2) the man had completed two semen analyses and was not hypogonadal or azoospermic; and (3) the woman had idiopathic primary or secondary infertility,

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explicitly defined as follows: the woman had (a) a spontaneous ovulatory cycle length variation of no more than 5 days: (b) adequate luteal phase, as demonstrated by a timed endometrial biopsy; (c) normal (<23 ng/mL) prolactin levels; (d) cervical mucus at midcycle with a spinnbarkeit of at least 6 cm and ferning of at least 3+ (on a scale of 1 to 4); and (e) no evidence of tubal or ovarian disease and minimal or no evidence of endometriosis, as shown by laparoscopy. (Ten women had minimal endometriosis.)

Control Subject Definition

Control subjects were recruited from prenatal classes at a private obstetrics practice that admitted to the same hospital as the infertility practices and was located in the same neighborhood. This practice performed approximately 1000 deliveries annually, and its composition was as follows: White, 80%; other, 20%. All patients seen in this practice were privately insured.

Men were enrolled as control subjects if they met the following criteria: (1) the couple had been in a stable union for at least 1 year; (2) this was the couple's first pregnancy; and (3) neither partner had undergone prior treatment for infertility.

Data Collection

The study was approved by the Institutional Review Boards of the study hospital and the health maintenance organization. Enrollment occurred between September 1987 and March 1989. After informed consent was obtained, case and control subjects completed a questionnaire on their medical and sexual histories and demographics. Medical records of the case subjects and their partners were abstracted by means of a standard protocol by a trained research nurse (K.H.) or physician (B.W., S.T.H.). Information necessary to ascertain the female partners' eligibility and results of the men's sperm analyses were recorded; only subjects for whom all information regarding entry criteria was explicitly stated were enrolled.

A blood sample was collected for antibody measurement and spun immediately, and serum was stored at −70°C until the conclusion of subject enrollment.

Laboratory

Serum IgG antichlamydia antibodies were measured in a single batch at the laboratory of one of the authors (J.S.) by an indirect microimmunofluorescent assay.[10] The reproducibility of the antichlamydia antibody assay (repeated measurements) in this laboratory was 95% ± one dilution. Samples were tested in a random order and laboratory personnel were blinded to the case-control status of the submitted samples.

Semen analyses were performed on the semen of case subjects only. A CellSoft (CRYO Resources Ltd, New York, NY) semen analysis system was used at the hospital site (65% of the analyses). One experienced andrology technician performed all analyses at the health maintenance organization site (35% of the analyses). The wide intrasubject variation in semen parameters requires that an average be used to approximate the true values over time.[11] Therefore, the average value of two semen analyses for each case subject was used to conduct an analysis of the relationship of sperm parameters to chlamydial antibody status.

Data Analysis

Univariate tests of significance were performed with the chi-square test of proportions or Fisher's Exact Test for dichotomous or polychotomous variables. The *t* test was used to compare differences between continuous variables.

We screened the data set for confounding in two ways. First, logistic regression analysis was used to test for variables that might confound the relationship between chlamydia and infertility; each candidate variable was added individually to a logistic model that contained only chlamydia antibody status (exposure) and case-control status (disease). A positive antibody status was defined as a titer of 1:64 or higher. A variable was considered a potential confounder if it altered the crude estimate by more than 10%.[12] Second, univariate tests of significance were performed to examine for relationships between candidate variables and infertility. Variables associated with infertility at the 0.25 level were considered possible confounders by this method.[12] Potential confounders identified by either logistic regression or univariate screening were entered into a multivariate logistic regression model to adjust the crude estimate of effect.

We also used logistic regression to perform a test for trend. Disease status (infertility) was modeled against the titer of antichlamydial antibody to preserve the geometric nature of the original data (i.e., the explanatory variable for antichlamydial antibody, treated continuously in the logistic model, was set to equal 0, 8, 16, 32, 64, 128, and 256 when the titer was 0, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256, respectively.) Confounding of this model was also assessed by the 10% change rule and univariate screening.

TABLE 1—Demographic Characteristics of Infertile Case and Fertile Control Subjects

	Case Subjects (n = 52)	Control Subjects (n = 79)	P[a]
Mean age when pregnancy attempt began, y			
Male	32	31	0.4
Female	31	31	0.9
Race			0.1
White	88%	93%	
Black	12%	5%	
Other	...	2%	
Education			0.6
High school	4%	9%	
Some college	11%	13%	
College	37%	29%	
Graduate school	48%	49%	
Household income			0.2
$21–40 999	10%	13%	
$41–60 999	32%	32%	
$61–80 999	29%	15%	
$81 000+	29%	40%	

[a]Unmatched *t* test (age) or chi-square test.

Results

A review of 675 charts of new and existing infertility patients yielded 55 eligible case subjects. Fifty-two (94%) agreed to participate. Of 108 men recruited at prenatal classes, 79 (73%) agreed to be controls. Demographic data were collected on 16 of the 29 men who refused participation. No significant differences were found between control participants and nonparticipants in age at initiation of pregnancy attempt, educational level, or income.

Characteristics of subjects are reported in Table 1. Case and control subjects and their partners were approximately the same age when attempts at conception began. Race, educational level, and household income were also similar. At the time of enrollment in the study, case subjects averaged 36 years of age and control subjects averaged 32 years of age ($P < .05$). Their partners averaged 35 and 31 years of age, respectively ($P < .05$).

TABLE 2—Relationship between Level of Antibody to *C trachomatis* and Male Infertility

Level of Antichlamydial Antibody Titer	No. Infertile Males (n = 52)	No. Fertile Males (n = 79)	Odds Ratio[a]	95% CI
≥1:8	28	41	1.1	0.5, 2.6
≥1:16	22	36	0.9	0.5, 1.6
≥1:32	19	25	1.2	0.6, 2.2
≥1:64	13	7	3.4	1.3, 9.1
≥1:128	5	1	8.3	1.3, 52.3
≥1:256	2	0	...	...

[a]Odds ratios are calculated on cumulative subgroups.

TABLE 3—Relationship between Genitourinary Diagnosis or Symptoms and Infertility Case–Control Status

	Case Subjects		Control Subjects		
	No.	%	No.	%	P^a
Genitourinary diagnosis					
Urethritis	2/49	4%	6/73	8%	0.4
Prostatitis	2/48	4%	3/73	4%	0.9
Gonorrhea	3/52	6%	4/79	5%	0.8
Any genitourinary diagnosis	7/52	13%	11/79	15%	0.4
Genitourinary symptoms					
Urethral discharge	5/52	9%	9/79	11%	0.7
Dysuria	20/52	38%	25/78	32%	0.4
Testicular symptoms	6/51	12%	10/79	13%	0.9
Any genitourinary symptom	23/52	44%	32/79	40%	0.7

[a]Chi-square test.

The numbers of case and control subjects who were antibody-positive for *C trachomatis* at each dilution are shown in Table 2. Infertile men were 3.4 times more likely than fertile men to have a higher titer (greater than or equal to 1:64) of chlamydia (odds ratio [OR] = 3.4, 95% confidence interval [CI] = 1.3, 9.1). If antibody titers of 1:8, 1:16, or 1:32 were used as cut-points, no increased risk was seen. A test-positive criterion of 1:128 yielded an OR of 8.3 (95% CI = 1.3, 52.3). The odds ratios presented in Table 2 were calculated by using the cumulative groupings that result from setting progressively higher antibody cut-points.

The analysis presented in Table 3 examined possible relationships between the men's genitourinary histories and infertility. In this sample, no statistically significant association between any previous diagnosis of genitourinary disease and infertility was observed, either in individuals or in the group. Similarly, self-reported genitourinary symptoms, alone or in combination, were not associated with male infertility.

As shown in Table 4, the sexual and contraceptive behaviors we measured were also unrelated to infertility. Case and control subjects reported similar numbers of previous sexual partners and patterns of use of barrier contraception. Frequency of intercourse was also similar in the two groups. Three of 50 case subjects and 7 of 73 control subjects reported having intercourse with another partner during the present relationship.

To check for confounding, each variable listed in Tables 1, 3, and 4 was entered singly into a logistic regression equation containing fertility status and presence of antichlamydia antibody at a titer of 1:64 or higher. No variable changed the estimate of effect by more than 10%. Additionally, because race and household income were associated (by univariate testing) with infertility at $\alpha = .25$ or less, they were simultaneously entered into a multivariate logistic regression model to control for confounding. The estimate of effect remained unchanged (adjusted OR = 3.4, 95% CI = 1.2, 9.2).

We performed a test of trend, using logistic regression, by modeling the titer of antichlamydial antibody against disease status. This test for trend uses nonoverlapping subgroups with the reference category of nonreactive antibody titer. The model was significant at $P = .046$ (Wald test), indicating a significant trend. The two variables associated with infertility at $P < .25$ (ethnic group and socioeconomic status) were added to this logistic model and the beta coefficient of the antibody titer remained significant at $P = .038$. The 10% change rule indicated that no other variables were potential confounders in the test for trend model.

Of the case and control subjects with antichlamydia antibody titers of 1:64 or higher (n = 20), 20% had experienced abnormal penile discharge, 20% reported nontraumatic testicular swelling or pain, and 40% recalled dysuria. Fifty percent of the antibody-positive subjects had never experienced any of these symptoms. Case and control subjects had similar rates of symptomatic infection and asymptomatic infection (i.e., serologic evidence of infection without reported symptoms).

No significant differences were found between semen characteristics of antibody-positive and antibody-negative infertile men (Table 5). However, the difference in mean sperm density between antibody-positive and antibody-negative case subjects (62.3 and 97.6 million, respectively) approached significance ($P = .1$).

Discussion

Although several lines of evidence suggest a role for *C trachomatis* in the etiology of male infertility, no direct link has been demonstrated.[6-8] Part of the difficulty in discerning this association relates to design challenges raised by the case-control study method. To assess the relationship between male infertility and chlamydia with a case-control paradigm, the following design points must be addressed: (1) male-factor infertility must be isolated as distinctly as possible; (2) a control population that approximates the population from which the case subjects arise must be identified; (3) an appropriate marker of exposure to *C trachomatis* must be defined; and (4) potential confounders of the relationship between chlamydia and male infertility must be sought and controlled for, if present.

Isolating male-factor infertility is the most difficult task. Theoretically, to be certain that the man is responsible for a

couple's infertility, one must determine that he is unable to impregnate a female of recently proven fertility who has sustained no intervening events that might diminish her fertility. This test of male infertility is obviously impractical. Our approach to capturing idiopathic male infertility relies on an exhaustive fertility evaluation of both partners that finds no cause. Thus, known female causes of infertility are excluded, and a group of males with idiopathic infertility is isolated. It must be noted that this definition of idiopathic male infertility could also serve, in its reciprocal form, as a definition of idiopathic female infertility. We chose to evaluate the male partners for an identifiable factor that might be linked to idiopathic infertility (i.e., exposure to chlamydia). Despite this limitation, the current study improves upon previous studies of male infertility that have not reported the fertility status of the female partners, that have stated that the female partners were "normal" but have not documented that fact, or that have used suboptimal criteria for the female evaluation.[6-8]

A cardinal feature of the case-control design is that the control subjects must be representative of the population from which the case subjects come.[13] In the present study, recruitment sites of case subjects and control subjects were chosen for their similarity in both demographic composition and geographic location. Control subjects were recruited from a population of first-time expectant fathers; the rationale was that first-time expectant fathers were most comparable in fertility potential to infertile males attempting a first pregnancy. Studies that have included men who may not have fathered children may have been biased toward the null by including potentially infertile males as "normal controls."[14] Further, we required that the control subjects have been in a stable union for at least 1 year before enrollment. This restriction partially addresses the problem of exposure bias (i.e., it helps to ensure that case and control subjects have had equal opportunity to be exposed to chlamydia).[2] As a further safeguard we also measured nonmonogamy. During the period when couples were attempting to conceive, if case subjects were less monogamous than control subjects, new exposures would be more likely to occur among case subjects. The rate of monogamy was approximately 94% among case subjects and 90% among control subjects.

A third key issue is the optimum choice for a marker of the exposure of interest. The indicator of past infection with *C trachomatis* was the presence of antichlamydia antibody at a titer of 1:64 or higher. A previous case-control study of male infertility in which one of our group (J.S.) participated revealed a high proportion of antibody-positive control subjects (57%) when a positive test was defined as a titer of 1:16.[8] One explanation for this is that low-level titers may be a marker for uncomplicated lower genital tract infection (urethritis) with no lasting sequelae, whereas higher titers may be associated with more serious infections (epididymitis) and important long-term sequelae such as infertility. Our primary analysis defined as "test-positive" dilutions of 1:64 or higher, and our conclusions regarding the association of chlamydia and infertility are predicated on this definition. Titers of 1:32 or less were not associated with infertility, whereas the risk increased to eightfold at titers of 1:128 or higher.

Evidence for a correlation between strength of titer and biologic importance has been found in studies of chlamydia and ectopic pregnancy.[15,16] Antichlamydia antibody titers higher than 1:64 have been the levels at which associations were found. A similar association between the severity of salpingitis and the strength of antichlamydia antibody titers has also been reported.[17,18] There is no general agreement in the literature, however, regarding the level of antichlamydia antibody that should be deemed important. In addition, antibody levels can wane with time; however, this occurrence would only bias the current study toward the null, making it more difficult to detect an effect. We used another approach, the test for trend, to evaluate the relationship between strength of antibody titer and infertility. The finding of a significant trend test (controlled for confounding) further supports the association between chlamydia and infertility. It suggests that the biological sequelae of the infection may be pro-

TABLE 4—Relationship between Sexual Practices and Infertility Case–Control Status

	Case Subjects		Control Subjects		
	No.	%	No.	%	P^a
Number of prior partners					0.6
1–5	27/51	53%	32/79	40%	
6–10	9/51	18%	18/79	23%	
11–20	5/51	9%	12/79	15%	
20+	10/51	20%	17/79	22%	
Use of condoms					
Ever vs never	38/52	73%	60/78	77%	0.6
Frequent[b] use short-term[c] vs infrequent use short-term	5/47	14%	11/74	15%	0.5
Frequent[b] use long-term[d] vs infrequent use long-term	4/49	8%	9/77	12%	0.5
Use of diaphragm					
Ever vs never	32/52	61%	52/79	66%	0.6
Frequent[b] use long-term[d] vs infrequent use long-term	18/52	35%	32/79	40%	0.5
Intercourse frequency					0.6
1–4 times/mo	18/52	35%	25/79	32%	
5–8 times/mo	19/52	36%	27/79	34%	
9–12 times/mo	12/52	23%	15/79	19%	
>12 times/mo	3/52	6%	12/79	15%	

[a]Chi-square test.
[b]More than 50% of sexual encounters.
[c]Short-term relationships, defined as those lasting ≤3 months.
[d]Long-term relationships, defined as those lasting >3 months.

TABLE 5—Semen Parameters of Chlamydia Antibody-Positive[a] Infertile Men Compared with Chlamydia Antibody-Negative Infertile Men

Mean Semen Parameters	Antibody Positive	Antibody Negative	P^b
Volume (cc)	2.7	2.2	0.3
Density (millions)	62.3	95.6	0.1
Motile (%)	52.9	51.7	0.9
Normal forms (%)	72.9	73.5	0.9

[a]Antibody titer ≥1:64.
[b]Unmatched *t* test.

portional to the strength of the immune response; this may well reflect the degree of inflammation and histological damage.

A confounder may falsely increase or decrease the true estimate of effect; it must be related to both disease (infertility) and exposure (male infection with chlamydia).[19] No known factors are definitely associated with both *C trachomatis* and male infertility, but several should be considered, including race, socioeconomic status, age at initiation of conception attempts, and history of gonococcal infection. The methodology we used to detect confounding included both univariate tests of association between each candidate confounder and infertility and a multivariate change in estimate technique. Univariate screening of the data set with a less stringent alpha level is perhaps most useful when little prior information is known about the existence of the tested associations.[12] However, in small data sets, type II error is likely even when a larger alpha is used. Additionally, this approach does not assess the association between the exposure and the candidate variable. Therefore, we employed a percentage change test to compare the crude estimate of effect to an adjusted estimate, accounting for each potential confounding variable. The crude estimate is retained if the odds ratios are sufficiently similar.[12,20]

Potential confounders included two that are known to be associated with chlamydial infection: race and socioeconomic status.[9] The seroprevalence of *C trachomatis* antibody may also increase with age (assuming nonmonogamy); thus, male age at initiation of conception attempts was considered. Because fecundity is influenced by female age at initiation of conception attempts, we assessed this factor as well.[21,22] The final likely candidate for confounding is previous male gonococcal infection. Because there is no good serologic test for previous gonococcal infection, historical markers (Table 3) were used. Because some historical markers for gonococcal infection may also be indicators of chlamydia exposure, this approach may be too conservative. Nevertheless, we found no evidence of confounding by these variables.

Given these findings, what pathophysiologic mechanism might be invoked to explain a threefold increased risk of infertility associated with a high chlamydia titer? The epididymis plays a critical and complex role in sperm maturation and transport, and effects on this structure may affect sperm function and therefore fertility. *C trachomatis* has been isolated by direct aspiration from the epididymis in symptomatic epididymitis; some authors have attributed 40% to 80% of epididymitis to chlamydia.[23] Effects of chlamydia on sperm characteristics in infertile subjects have been sought, but have not been found.[6,24] Our findings are suggestive of an effect on sperm number, but the difference between the lower mean sperm density seen in the antibody-positive case subjects and the higher density in the antibody-negative case subjects did not reach statistical significance.

Chlamydia has been associated with female tubal infertility, and studies in women have consistently reported a smoldering or asymptomatic character of the infection.[25] Up to 25% of women with tubal infertility attributed to chlamydia did not recall any symptoms.[18,26] We wonder whether a similar subtle presentation occurs in men; only 50% of the antibody-positive men ever experienced any symptom consistent with this infection.

No relationship between male infertility and genitourinary symptoms, genitourinary diagnoses, or sexual practices (e.g., barrier method use) was demonstrated. Because these comparisons were made to assess for confounding in our data set, one should not conclude that no association exists between these factors and infertility in the population as a whole.

We found an association of idiopathic bilateral infertility with antibody to *C trachomatis* in the male partner. Our hypothesis, that male-factor infertility is associated with chlamydial infection, is consistent with these results. The conclusion that the association between chlamydia and idiopathic infertility demonstrated here can be assigned to the male partner cannot be definitively made in the context of this study design. We did not measure female serologies; one could argue that idiopathic female infertility is associated with chlamydial exposure and that the male serologies are markers for this association as well.

The establishment of a causal link between chlamydia and infertility in men would have notable consequences for public health policy. Previous evaluations of the cost-effectiveness of screening for *C trachomatis* infection have recommended that screening not be undertaken in men, in part because of the lack of evidence for serious long-term sequelae such as infertility.[27] The existence of such sequelae would necessitate the reevaluation of these recommendations. The potential importance of screening programs is underscored by our finding of serologic evidence for a 50% rate of asymptomatic infection.

Thus, we feel our findings suggest the need for further research. A study of a similar idiopathically infertile group of couples in whom both partners' serologies are obtained may be helpful in distinguishing whether the association of chlamydia and infertility we observed can truly be ascribed to the male partner. □

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