Mycoplasma genitalium, *Chlamydia trachomatis*, and tubal factor infertility—a prospective study

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Objective: To determine the presence of *M. genitalium* and *C. trachomatis* in women attending fertility clinics and to follow these women for the effects of previous infections or tubal damage on pregnancy rate and outcome.
Design: Prospective study.
Setting: Fertility clinics and university.
Patient(s): Two hundred twelve couples attending fertility clinics.
Intervention(s): Blood and cervical swab samples from the women. Tubal status was assessed by culdoscopy and/or laparoscopy.
Main Outcome Measure(s): Presence of *M. genitalium* and *C. trachomatis* was determined by polymerase chain reaction. Serum samples were tested for antibodies against *M. genitalium* and *C. trachomatis*.
Result(s): One swap sample was positive to *C. trachomatis* and none positive to *M. genitalium*. Thirty of the 194 women had tubal factor infertility (TFI); 23% and 17% of women with TFI had antibodies to *C. trachomatis* and *M. genitalium*, respectively, compared with 15% and 4%, respectively, of women with normal tubes; 36% and 14% of women with a self-reported history of pelvic inflammatory disease (PID) were seropositive to *C. trachomatis* and *M. genitalium*, respectively, compared with 10% and 6%, respectively, of women without past PID.
Conclusion(s): A strong antibody response against *M. genitalium* or *C. trachomatis* but no sign of current or chronic infection was found in women with TFI, indicating that previous infections caused by these microorganisms may have resulted in permanent damage and occlusion of the fallopian tubes. (Fertil Steril® 2008;90:513–20. ©2008 by American Society for Reproductive Medicine.)

Key Words: Culdoscopy, LightCycler PCR, *Mycoplasma genitalium*, serology, tubal infertility

Chlamydia trachomatis and *Mycoplasma genitalium* are sexually transmitted bacteria causing urogenital diseases in both men and women. In fact, *C. trachomatis* is one of the most prevalent causes of sexually transmitted infections globally (1). *Chlamydia trachomatis* is the major cause of mucopurulent cervicitis (2), but an estimated 70%–75% of the infections are asymptomatic. Approximately 20% of the *C. trachomatis* lower genital tract infections develop into pelvic inflammatory disease (PID) (3). The long-term sequelae of PID can result in ectopic pregnancy, infertility, and chronic pelvic pain (4, 5). *Chlamydia trachomatis* has been shown to cause salpingitis followed by tubal occlusion in grivet monkeys (6) and was isolated directly from women with acute salpingitis (7). Seroepidemiologic studies indicate that *C. trachomatis* may be responsible for a major part of the cases of tubal factor infertility, ectopic pregnancy, and adverse pregnancy outcome (8).

Mycoplasma genitalium is a frequent cause of male nongonococcal urethritis (NGU), and evidence that *M. genitalium* is sexually transmitted has been shown by high prevalence rates of 56% among male sexual partners of infected women (14) and 32% among the female partners of infected men with NGU (35). Furthermore, data showing *M. genitalium* as a cause of genital tract diseases in women are emerging (9). Since the first isolation of *M. genitalium* in 1980 the major hindrance for studying its role in human diseases has been that it is very difficult to cultivate and no commercial serology test is available (10, 11). Attempts to culture *M. genitalium* from patient specimens have failed mainly because of its slow-growing nature, taking months to induce a color change of the SP-4 medium. Consequently, there is a high risk of overgrowth by other microorganisms, e.g., *Mycoplasma hominis*, that grow well in SP-4 medium (12). Polymerase chain reaction (PCR) has been the most successful tool to identify *M. genitalium*. Serology methods are also available, although cross-reactivity to the closely related *M. pneumoniae*, the cause of atypical pneumonia, is observed (11, 13).

Recent PCR studies associate *M. genitalium* with female lower and upper genital tract diseases in both industrialized

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and developing countries. *Mycoplasma genitalium* was detected in 7% of women attending a sexual disease clinic in Seattle, Washington, and those women infected with *M. genitalium* had a 3.3 times greater risk of mucopurulent cervicitis (14). A Swedish study showed a frequent occurrence of *M. genitalium* in women with lower genital tract infections, with 50% of the *M. genitalium*–infected women having signs and symptoms of urethritis and/or cervicitis (15). The prevalence of *M. genitalium* among female sex workers in West Africa was high, with 26.3% being positive, and the association with cervicitis was statistically significant with odds ratios (OR) of 1.6–1.8 (16). The presence of *M. genitalium* in the cervix and/or endometrium of women from Nairobi, Kenya, with histologically confirmed acute endometritis was 16% (9/58) compared with 2% (1/57) of women without endometritis (17). A British study linked *M. genitalium* to PID, where 13% (6/45) of women with clinically diagnosed PID were *M. genitalium* PCR positive compared with none in the control group (18). Based on the evidence that *M. genitalium* can colonize and exist in the upper genital tract of women with PID, it is likely that it can spread to the fallopian tubes and cause damage there.

Mycoplasma genitalium appears to be associated with these diseases independently of *C. trachomatis* and *Neisseria gonorrhoea.* In a study from Kenya, 7% (9/123) of women with a laparoscopically diagnosed salpingitis were PCR positive to *M. genitalium*, but four were coinfected with *C. trachomatis* (19). Furthermore, in a retrospective study we demonstrated a correlation between IgG against the immunodominant adhesion protein, MgPa, of *M. genitalium* and women with tubal factor infertility (TFI), with an OR of 3.8 (20). This association was independent of antibodies to *C. trachomatis*, and the prevalence of *M. genitalium* IgG in women with TFI was 22% (29/132) versus 6% (11/179) in women with normal tubes.

The presence of *M. genitalium* in women with TFI, however, has not been investigated and was one aim of the present study. Serum and swab specimens from the female partner of 212 couples as they were seeking help for infertility at two Danish fertility clinics were analyzed for antibodies and presence of *M. genitalium* and *C. trachomatis* by PCR. The tubal status was assessed by culdoscopy/laparoscopy, and other clinical data about previous PID, infertility of the male partner, pregnancy rate, and pregnancy outcome were recorded.

MATERIALS AND METHODS

Patients

During the years 2002–2005, serum and cervical swab specimens were collected from 212 infertile women attending the Horsens-Brædstrup or the Holstebro fertility clinic in Denmark. The study was approved by the local scientific ethical committees (journal no. 20010161), and written consent was obtained from each patient.

The women were consecutively recruited for the study and only included if culdoscopy (21, 22) and/or laparoscopy was indicated. Insufflation with methylene blue was performed to investigate for tubal passage. Immediately before the examination for tubal patency, the uterine cavity of the women was examined by hysteroscopy. The women were followed prospectively over a mean time period of 20 months. At the initial visit, blood and cervical swab samples were obtained from the woman to examine for previous or current infections with *M. genitalium* and *C. trachomatis*. In addition, the sperm quality of the male partner was examined.

Semen analysis was carried out according to World Health Organization criteria (1992). The semen samples were collected by masturbation after at least 3 days of abstinence. Semen volume was determined, and sperm motility and morphology were measured in raw serum as well as after PureSperm (Nidacon International, Göteborg, Sweden) gradient purification.

The couples were classified into one or more of the following categories: 1) TFI, due to lack of passage through one or both tubes as verified by culdoscopy or laparoscopy; 2) male factor infertility with severely reduced semen quality, i.e., less than 5 million/mL motile morphologically normal sperm in the raw semen or less than 600,000 motile sperm after PureSperm gradient centrifugation (i.e., the indication for intracytoplasmic sperm injection [ICSI]); 3) anovulation, comprising women with amenorrhea or oligomenorrhea; 4) endometriosis verified by culdoscopy/laparoscopy; or 5) unexplained infertility, in the case of normal results of culdoscopy/laparoscopy, semen analysis, and regular menstruation cycles.

At the primary visit, the women were asked whether they had suffered from previous episodes of PID and whether they had received any antibiotic treatment for the PID, including treatment for *C. trachomatis*. Other clinical data about age, spontaneous and treatment-related pregnancies, and pregnancy outcomes was recorded at the ending of the study period.

Collection of Clinical Specimens

The serum and endocervical swab samples were obtained before intrauterine insemination, in vitro fertilization (IVF), or intracytoplasmatic sperm injection was initiated. The *M. genitalium* swab specimen was taken just before the routine *C. trachomatis* swab specimen. Routine detection of *C. trachomatis* was done by Amplicor (Roche Diagnostic Systems, Branchburg, NJ) at Vejle Hospital, Denmark.

For detection of *M. genitalium*, endocervical specimens were taken with sterile *C. trachomatis* swabs (Amplicor; Roche Diagnostic Systems) and inoculated into plastic tubes containing 2 mL SP-4 medium (23) with 0.01% thallium acetate. The samples were sent to the Institute of Medical Microbiology and Immunology, University of Aarhus, Denmark, where swabs were stored at −70°C. Blood samples were placed at 4°C overnight before the coagulum was removed. The samples were centrifuged at 650*g* for 15 minutes

to extract the serum supernatant. The serum was stored at −70°C.

Mycoplasma genitalium Culture

Mycoplasma genitalium G37 (American Type Culture Collection) was cultured in 100 mL SP-4 medium (23) in 150-mL tissue culture flasks (TPP, Trasadingen, Switzerland) and incubated at 37°C. After 48 hours, the *M. genitalium* culture was harvested in the exponential growth phase, which was indicated by a change in the color of the medium from red to orange. The SP-4 medium was poured off, and the *M. genitalium* colonies attached to the bottom were scraped off in phosphate-buffered saline (PBS: 10 mmol/L sodium phosphate, 145 mmol/L NaCl, pH 7.4) and pelleted by centrifugation at 15,000*g* for 45 minutes. The cells were resuspended in 4 mL PBS and centrifuged in Eppendorf tubes at 20,000*g* for 20 minutes. The supernatant was discarded and the pellets stored at −70°C.

DNA Extraction from Swab Samples

Three hundred microliters of the swab sample was centrifuged at 20,000*g* for 30 minutes, and the pellet was thoroughly washed twice with PBS. The DNA was released with proteinase K (Roche Diagnostic, Mannheim, Germany) treated by solubilizing the pellet in 50 μL TE buffer (10 mmol/L Tris(hydroxymethyl)aminomethane and 1 mmol/L EDTA) containing proteinase K (200 μg/mL). Proteins were digested at 55°C for 1 hour followed by enzyme inactivation for 10 minutes at 98°C.

To minimize the risk of contamination, the DNA extractions were performed in separate rooms away from *M. genitalium* culturing and general DNA preparation. Filter pipette tips were used for all DNA handling. The proteinase K–treated swab samples were stored at 4°C before the PCR analysis.

Human LightCycler PCR Assay

To examine the homogeneity of DNA in the proteinase K–treated samples we determined the load of human DNA by quantitative LightCycler PCR. This LightCycler assay was originally designed for amplification of both the mouse (24) and the human GAPDH gene (25). Real-time PCR was performed as described previously by Mygind et al. (26).

Mycoplasma genitalium LightCycler PCR Assay

The proteinase K–treated specimens were tested by the quantitative LightCycler PCR assay using the *M. genitalium gap* gene as recently described in Svenstrup et al. (27). To investigate for PCR inhibition 20 negative proteinase K–treated swab specimens were randomly selected for a spiking assay. The samples were spiked with 100 copies of *M. genitalium* standard DNA before subjected to LightCycler PCR. To determine the PCR efficiency in the samples, the resulting PCR curves were fitted with a four-parametric sigmoid model using SARA version 1.0 (http://www.gram.au.dk) as described previously (27).

Immunoblotting

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting with whole cells of *M. genitalium* as antigen was described previously (20). The immunologic reactions to the MgPa protein of 156 kDa were graded as absent, weak, medium or strong. The intermediate and strong reactions were evaluated as positive samples whereas weak reactions were counted as negative samples, as described previously (20). The immunoblotting readings were performed without knowledge of the patient's status regarding PCR and clinical findings.

Chlamydia trachomatis ELISA

A commercial *C. trachomatis* ELISA (Medac, Hamburg, Germany) based on a synthetic peptide from the immunodominant region of the major outer membrane protein was used to examine the level of anti–*C. trachomatis* IgG antibodies in the patient serum. The test was performed in accordance with the manufacturer's instructions.

Statistical Methods

Fisher exact test was used to determine the significance of the difference between two independent groups. Associations between the presence of *M. genitalium* and/or *C. trachomatis* IgG antibodies and clinical findings were analysed by univariate and multivariate logistic regression. $P<.05$ was considered to be significant.

RESULTS

Infertile Couples

A total of 212 women were referred to examination by culdoscopy/laparoscopy before any further pregnancy planning. Before the operation, 18 women achieved spontaneous pregnancy (n = 9) or dropped out for personal reasons (n = 9). Therefore, the tube status was assessed in a total of 196 women. The 18 women were not included in the analysis regarding clinical infertility subgroups but were included in the PID and pregnancy outcome analysis.

The clinical infertility factors are shown in Table 1. Women with TFI (n = 30) comprised 14% of the studied group. Most couples (36%) suffered from male infertility. Among these, 44 men showed a severely reduced sperm quality, two of whom had Klinefelter syndrome. Women suffering from endometriosis (n = 18) accounted for a total of 9%. Anovulating infertile women (n = 45) constituted 21%, of which 16 (36%) were diagnosed with polycystic ovarian syndrome. A subgroup of couples with no clinical explanation for their infertility (n = 64), denoted unexplained infertility, made up 31%. The women's years of birth were recorded and the mean age was determined to be 30.0 ± 4.0 years (mean ± SD).

TABLE 1

Infertility factors, treatments, and pregnancies.

	No. of couples	%
Infertility factor	212	100.0
Not specified[a]	18	8.5
Tubal factor	30	14.2
Male factor	77	36.3
Severe male factor	44	20.8
Endometriosis	18	8.5
Anovulation	45	21.2
Unexplained	64	30.9
Normal tubes	164	77.3
Treatments		
IVF	53	25.0
ICSI	16	7.5
AIH or AID	117	55.2
Archived pregnancy	138	65.1
Spontaneously	42	30.4
Result of treatment	96	69.5

Note: IVF = in vitro fertilization; ICSI = intracytoplasmic sperm injection; AIH = artificial insemination with husband's sperm; AID = artificial insemination with donor sperm.

[a] Includes couples who conceived before the clinical investigation was completed or couples who decided not to continue the examinations.

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Evaluation of Sample Preparation Including a Spike Assay

To examine the DNA homogeneity of the prepared swab specimens, the human DNA load was measured by a quantitative LightCycler PCR developed by Mygind et al. (26), and spike assays monitored the level of inhibition. Two swab specimens were not received at the laboratory.

Human DNA was detected in all 210 samples, with a mean concentration of 1.7×10^3 copies/μL (SD 1.8×10^3, SEM 126, range 0.3–9.5 $\times 10^3$ copies/μL), and 7.9% of the samples contained fewer than 100 copies/μL of human DNA.

Twenty swab specimens were selected at random for a spiking assay. The samples were spiked with 100 copies/μL of purified genomic *M. genitalium* G37 DNA, and they could all be detected by the LightCycler PCR. The r^2 values were all higher than 0.999, and the mean slope (b) value was 1.30 (SD 0.07, SEM 0.02), which was not significantly different from the b value of the standard curve (b = 1.33; P=.087). Thus, the PCR efficiency was found to be acceptable.

Detection of *M. genitalium* and *C. trachomatis* by PCR

One cervical swab (0.5%) of 212 swab samples tested by the *C. trachomatis* routine PCR was found to be positive. The serum sample obtained from the PCR-positive woman was seronegative to *C. trachomatis* by ELISA. Moreover, the patient did not have a history of PID, and her fallopian tubes were normal.

The 210 proteinase K–treated swab specimens were tested in doublets for the presence of *M. genitalium* by LightCycler PCR. One sample (patient no. 16) was found positive in only one of the doublets, with a concentration of 2.5 copies/μL; but three additional doublet tests failed to detect *M. genitalium* DNA, so the sample was regarded as negative.

After having controlled for PCR inhibition and efficiency, we concluded that none of 210 swab specimens were PCR positive for *M. genitalium*.

Prevalence of *M. genitalium* and *C. trachomatis* IgG Antibodies and Distribution by Clinical Subgroups

Serum samples obtained from the 212 women were tested for *M. genitalium* antibodies against MgPa by immunoblotting and for *C. trachomatis* antibodies by the pELISA test (Medac). The total seroprevalence of *M. genitalium* and *C. trachomatis* were 7% (15/212) and 15% (31/212), respectively. Coexistence of antibodies against *M. genitalium* and *C. trachomatis* is shown in Table 2. Five women had antibodies to both pathogens. Among the five women with antibodies to both microorganisms, two had developed TFI. Three women with TFI who were seronegative to *C. trachomatis* had antibodies to *M. genitalium*.

The frequency of MgPa antibodies in the group of women with TFI was higher than in any of the other clinical groups, with a rate of 17% (5/30) compared with 4% (7/164) in women with normal tubes (Fig. 1).

Using logistic regression we analyzed the association of antibodies to *M. genitalium* or *C. trachomatis* with TFI, male factor infertility, anovulation, and endometriosis. The results are shown in Table 3. Among the 194 women with a determined tubal status, the association between women with TFI and women seropositive to *M. genitalium* was significant, with an OR of 4.5 (95% confidence interval [C]) 1.3–15.2; P=.016). There was no association of the other clinical factors, but women with antibodies to *M. genitalium*

TABLE 2

Coexistence of *M. genitalium* (MG) and *C. trachomatis* (CT) antibodies.

MG/CT antibodies	No. of women with TFI	Total
+/+	2	5
+/−	3	10
−/+	5	26
−/−	20	171
Total	30	212

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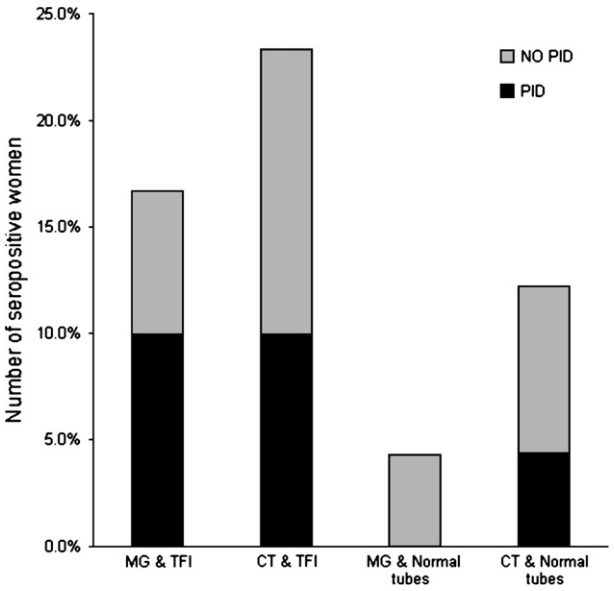
FIGURE 1

Prevalence of *M. genitalium* and *C. trachomatis* antibodies among women with tubal factor infertility (TFI) and normal tubes. The proportion of women with a self-reported previous PID is marked by the solid part of the columns.



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and a partner with severe male infertility almost significantly correlated with MgPa seropositivity (OR 2.7, CI 0.9–8.0: P=.074).

Of the women with TFI, 23% (7/30) had antibodies to *C. trachomatis* compared with 15% (24/164) of the women with normal tubes (Fig. 1). The correlation, however, between women with TFI and women seropositive to *C. trachomatis* was not statistically significant (OR 2.2, CI 0.8–5.8; P=.111), and no other clinical factors were associated with *C. trachomatis* seropositivity (Table 3). To investigate the antibody relation between the two pathogens and their association to TFI and male infertility, multivariate regression analysis was performed. The resulting ORs and CIs are shown in Table 4. After adjusting for *C. trachomatis* antibodies and age, *M. genitalium* antibodies were shown to be independently and significantly associated with TFI (OR 4.5, CI 1.2–15.6; P=.022). This was not the case for *C. trachomatis* antibodies, which after adjustment for *M. genitalium* antibodies and age was not significantly associated with TFI (Table 4).

Prevalence of *M. genitalium* and *C. trachomatis* IgG Antibodies in Women With a Previous PID Infection

The prevalence of antibodies to *M. genitalium* and *C. trachomatis* among women with a history of PID is summarized in Figure 1. Of the 212 women, 36 (17%) reported having suffered from a previous PID infection requiring antibiotic treatment. The prevalence of *C. trachomatis* IgG antibodies in women with a history of PID was 36% (13/36) compared with 10% (18/176) in women without PID. The difference was statistically significant, with a P value of $<.001$. Of the women with a previous PID, 24 reported to have received treatment for a *C. trachomatis* infection. Among these, nine (38%) had antibodies to *C. trachomatis* (Table 5A).

TABLE 3

Association of clinical infertility factors to the presence of *M. genitalium* (MG) or *C. trachomatis* (CT) antibodies in the female serum sample using univariate logistic regression.

	MG- CT-	MG+	OR[a] (95% CI)	*P* value	CT+	OR (95% CI)	*P* value
TFI							
Yes	20 (9.9%)	5 (33.3%)	4.5 (1.3–15.2)	.016	7 (22.6%)	2.2 (0.8–5.8)	.111
No	182	10			24		
Male factor							
Yes	59 (30.6)	7 (46.7%)	1.6 (0.5–4.5)	.414	13 (41.9%)	1.3 (0.6–2.8)	.521
No	134	8			18		
Severe male factor							
Yes	32 (16.1%)	6 (40.0%)	2.7 (0.9–8.0)	.074	8 (25.8%)	1.3 (0.6–3.3)	.501
No	167	9			23		
Endometriosis							
Yes	15 (7.2%)	1 (6.7%)	0.8 (0.1–6.1)	.793	2 (6.6%)	0.7 (0.2–3.3)	.661
No	194	14			29		
Anovulation							
Yes	38 (18.5%)	3 (20.0)	0.9 (0.2–3.4)	.904	4 (12.9%)	0.5 (0.2–1.5)	.227
No	167	12			27		

Note: OR = odds ratio; CI = confidence interval; TFI = tubal factor infertility.

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TABLE 4

Association of *M. genitalium* and *C. trachomatis* to clinical findings using multivariate regression analysis.

	M. genitalium		*C. trachomatis*	
	AOR (95% CI)	***P* value**	**AOR (95% CI)**	***P* value**
TFI	4.5 (1.2–15.6)	.022	1.9 (0.7–5.2)	.206
Male factor	1.5 (0.5–4.3)	.481	1.3 (0.6–2.8)	.554
Severe male factor	2.6 (0.9–7.8)	.092	1.2 (0.5–3.0)	.673

Note: AOR = adjusted odds ratio (the odds ratios were adjusted for the presence of antibodies against the other bacteria and for age); CI = confidence interval; TFI = tubal factor infertility.

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The seroprevalence of *M. genitalium* antibodies in women with a PID history was 14% (5/36) compared with 6% (10/176) of the women without PID (Table 5A). The difference, however, did not reach statistical significance (*P*=.143).

Four of the women reporting previous PID had antibodies to both microorganisms (Table 5A). Women with a self-reported history of PID and seropositivity to *M. genitalium* and/or *C. trachomatis* are compared with women with TFI in Table 5B. Of the 194 women examined with culdoscopy/laparoscopy, 33 reported previous PID, among which eight (24%) had TFI and five of those eight reported having been treated for *C. trachomatis*. Of the eight TFI women reporting previous PID, two were seropositive to both *C. trachomatis* and *M. genitalium*, and an additional two were seropositive to each of the two pathogens, respectively (Table 5B). Three of the five women with TFI and previously treatment for a *C. trachomatis* upper genital tract infection had antibodies to *M. genitalium* (Table 5B).

None of the women with normal tubes and a self-reported history of PID had antibodies to *M. genitalium*, but two women with antibodies to *M. genitalium* and a history of PID were never examined by culdoscopy/laparoscopy. In contrast, eight women with a history of PID and normal tubes were seropositive to *C. trachomatis*. Of women with a history of PID and normal tubes, 78% (7/9) reported having received treatment for a *C. trachomatis* infection.

Pregnancy and Pregnancy Outcome

During the study period, 138 of 212 women achieved pregnancy. A total of 42 (30%) pregnancies were achieved spontaneously whereas 96 (70%) were the result of treatment (Table 1). When the project ended, 119 children had been born and 23 women were still pregnant.

Of the treatment-related pregnancies, 47 were a result of artificial insemination by partner or donor (AIH/AID) and

TABLE 5

Prevalence of antibodies to *C. trachomatis* (CT) and *M. genitalium* (MG) in women with previous PID (A) and in relation to women with tubal factor infertility (TFI) (B).

	Previous PID							
	PID total		CT-treated PID		No PID		Total	
A								
C. trachomatis (IgG+)	13	36.1%	9	37.5%	18	10.2%	31	14.6%
M. genitalium (IgG+)	5	13.9%	3	12.5%	10	5.7%	15	7.1%
CT + MG (IgG+)	4	11.1%	2	8.3%	1	0.6%	5	2.4%
Total no. of women	36	17.0%	24	11.3%	176	83.0%	212	100.0%
B								
C. trachomatis (TFI + IgG+)	3	9.1%	2	8.7%	4	2.5%	7	3.6%
M. genitalium (TFI + IgG+)	3	9.1%	3	13.0%	2	1.2%	5	2.6%
CT + MG (TFI + IgG+)	2	6.1%	2	8.7%	0	0.0%	2	1.0%
Total no. of women with TFI	8	24.2%	5	21.7%	22	13.7%	30	15.5%
Total no. of women examined by culdoscopy/laparoscopy	33	17.0%	23	11.9%	161	83.0%	194	100.0%

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49 of IVF/ICSI. Among women treated with AIH/AID, 4% were seropositive to *M. genitalium* and 15% seropositive to *C. trachomatis*. Likewise, 4% of the women treated with IVF/ICSI were seropositive to *M. genitalium* and 20% seropositive to *C. trachomatis*. However, there was no statistical significant difference in the use of various ART techniques between these groups and no difference between *C. trachomatis* or *M. genitalium* seropositive or seronegative women and healthy pregnancy outcome. A difference of 15% *C. trachomatis*–seropositive miscarrying women and 5% seronegative miscarrying women did not reach statistical significance (P=.074). None of the women with adverse pregnancy outcome had antibodies to *M. genitalium*.

DISCUSSION

Present Infections With *M. genitalium* and *C. trachomatis*

None of the cervical swab specimens from women with TFI or normal tubes were PCR positive to *M. genitalium*, and only one woman was PCR positive to *C. trachomatis*, and her tubes were normal. In Denmark, the incidence of *C. trachomatis* is highest among women aged 15–24 years (28). Because the women of our study are older (mean 30 years) and live in steady relationships, they are less likely to be *C. trachomatis* positive. The PCR-positive rate of *C. trachomatis* (0.5%) found in the present study suggests low *C. trachomatis* prevalence in patients attending the two Danish fertility clinics. For comparison, a recent work from Sweden reports a significantly higher *C. trachomatis* DNA prevalence of 6.8% in a similar prospective study (29).

No other published studies have investigated the *M. genitalium* prevalence in specimens from women attending fertility clinics for family planning, but in another Danish study *M. genitalium* was found in 0.9% and *C. trachomatis* in 1.5% of the semen samples obtained from 209 men attending a fertility clinic (30).

Previous Infections With *M. genitalium* and *C. trachomatis*

This prospective study showed a significant correlation between antibodies against *M. genitalium* and women with TFI independent of *C. trachomatis* seropositivity. These findings were similar to the results of our previous retrospective study (20), despite the different design of the two studies. The presence of IgG antibodies against *C. trachomatis* was associated with PID but, surprisingly, not significantly associated with TFI. The lack of statistical significance may be explained by the limited number of women with TFI in the present study and by an increased focus on treatment and prevention of chlamydial infections in Denmark since the mid-1980s. Screening studies have shown that PID is less likely to occur among women treated for *C. trachomatis* infection (31, 32). Therefore, the enhanced diagnostic and antibiotic treatment of *C. trachomatis* infections in the 1990s may have resulted in fewer women with TFI and *C. trachomatis* antibodies as observed in the present study.

It is interesting that two of the *C. trachomatis*–seropositive women with TFI and a history of treated PID were seropositive to *M. genitalium* as well, because a recent open pilot study showed that only a prolonged treatment with azithromycin (500 mg day 1 and 250 mg days 2–5) is effective against male urethral infections (33). The single-dose azithromycin and other antibiotics frequently and effectively used against *C. trachomatis* infections appear not to be sufficient to eradicate *M. genitalium* from men with urethritis (33, 34), and in a Kenyan study it was shown that *M. genitalium* infections were persistent in female sex workers despite frequent use of antibiotics, including broad-spectrum antibiotics (35).

According to the present study it can not be ruled out that *M. genitalium* is an innocent bystander of PID leading to TFI, but along with its resistance and persistent nature, *M. genitalium* has a potential to be an important cofactor and/or the actual cause of TFI. This is supported by the ability of *M. genitalium* to attach to the ciliated epithelium of the human fallopian tubes (36). The pathogenic potential of *M. genitalium* to cause serious upper genital tract disease was also demonstrated in a study with five experimentally *M. genitalium*–infected monkeys. All five animals produced a moderate to severe endosalpingitis (37). In the Kenyan study of women with salpingitis (19), *M. genitalium* was detected in one fallopian tube specimen. That study was the first to actually detect *M. genitalium* in the fallopian tubes. The route of infection has not yet been established, but most likely *M. genitalium* reaches the tubes by colonization of the lower genital tract and from there spreads into the upper genital tract. However, in a previous study we have shown that *M. genitalium* can attach to motile spermatozoa and then may hitch-hike to the female upper genital tract (38).

There was no difference in the spontaneous or treatment-related pregnancy rates between women with or without serological evidence of a past infection with *M. genitalium* or *C. trachomatis*. Several studies have addressed this issue regarding *C. trachomatis* and found no differences (29, 39, 40). In conclusion, none of the swab samples were PCR-positive to *M. genitalium* or *C. trachomatis* except for one *C. trachomatis* PCR–positive swab. Accordingly, there was no sign of current or chronic *M. genitalium* or *C. trachomatis* infection. Despite this, we demonstrated an antibody response primarily in women with TFI. Thus, these results emphasize the importance of studying the role of these two pathogens in acute PID and long-term sequelae.

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