

Chlamydia trachomatis, Ureaplasma urealyticum and Mycoplasma hominis urogenital infections in patients with infertility. Results from a large cross-sectional study.

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Research Article

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Abstract

Female and male infertility have been associated to *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis* urogenital infections. However, evidence from large studies assessing their prevalence and putative associations in patients with infertility is still needed. The study design was a cross-sectional study including 1554 female and 3610 male patients with infertility in Cordoba, Argentina. In women, the prevalence of *C. trachomatis*, *U. urealyticum* and *M. hominis* urogenital infection was 4.3%, 31.1% and 12.1% whereas in men it was 5.8%, 19.2% and 5.3%, respectively. *C. trachomatis* infection was significantly more prevalent in men whereas *U. urealyticum* and *M. hominis* infections were more prevalent in women. Besides, *C. trachomatis* and *U. urealyticum* infections were significantly higher patients younger than 25 years. Moreover, *U. urealyticum* and *M. hominis* infections were associated to each other in either infertile women or men. Our data revealed that *C. trachomatis*, *U. urealyticum* and *M. hominis* are prevalent uropathogens in infertile patients. Of clinical importance, *C. trachomatis* and *U. urealyticum* infections were more prevalent in young patients whereas *U. urealyticum* and *M. hominis* are reciprocal risk factors of their co-infection. These results highlight the importance of including the screening of urogenital infections in the diagnostic workup of infertility.

Introduction

Urogenital infections are known causes of infertility¹. Currently, infertility affects 15–20% of reproductive-aged couples worldwide and women and men equally contribute to infertility cases^{1,2}. Sexually transmitted infections can impair fertility by different mechanisms: by directly damaging organs and gametes and/or, indirectly, by the induced inflammation and associated tissue damage, scarring and obstruction^{1,3}. Moreover, infection-induced genital inflammation may alter the normal immunomodulation process that naturally occurs in the female genital tract after mating to facilitate fertilization, embryo implantation and promote embryo growth for a successful pregnancy⁴. Besides being the most frequent sexually transmitted bacterial infection worldwide, *Chlamydia trachomatis* is a common infection associated to infertility⁵. In women, *C. trachomatis* is a known cause of different urogenital pathologies such as acute urethritis, cervicitis and salpingitis that may lead to severe reproductive complications including pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy, miscarriage and tubal infertility^{6,7}. In men, *C. trachomatis* is considered the most common agent of non-gonococcal urethritis and may cause epididymitis-orchitis, prostatitis, sperm tract obstructions and alterations in sperm quality^{8,9}. On the other hand, *Mycoplasma hominis* and *Ureaplasma urealyticum* have also been recognized as sexually transmitted infections that could impair human fertility¹. Although they are both known to colonize the female and male reproductive tracts as commensals, cumulative growing evidence has shown they are emerging sexually transmitted opportunistic pathogens able to cause asymptomatic chronic disorders affecting female and male fertility^{10–15}. In men, *U. urealyticum* and *M. hominis* are causes of non-gonococcal urethritis contaminating semen during ejaculation. Moreover, *U. urealyticum* has been proposed to cause prostatitis, epididymitis and infertility¹. In addition, reported data have shown that both *U. urealyticum* and *M. hominis* could impair sperm quality^{11,16,17}. In women, *U. urealyticum* and *M. hominis* may cause different pathologies including acute urethritis, bacterial vaginosis, pelvic inflammatory disease and tubal infertility^{14,18,19}. Moreover, the asymptomatic colonization by mycoplasmas or ureaplasmas could induce pro-inflammatory immune responses in the endometrium that may impair pregnancy outcomes^{2,9,14,20}.

The detection rates of *C. trachomatis*, *U. urealyticum* and *M. hominis* in the urogenital tract form infertile women and men has shown striking variations across regions and countries and in different groups when individuals were classified according to age, ethnicity and socioeconomic status^{5,21–23}. In that regard, a growing number of studies have been reported during the last decade. However, compelling available data from large cross-sectional studies is scarce^{21–23}. Moreover, reported data about the association of these infections in either infertile women or men is limited^{21–23}. Since these infections may play a significant role in the etiology of infertility, we herein conducted a large observational investigation into urogenital *C. trachomatis*, *U. urealyticum* and *M. hominis* infections in infertile women and men seeking care for infertility. Moreover, we analyzed the associations among infections and with demographic parameters such as patient sex and age.

Results

Prevalence of *C. trachomatis*, *U. urealyticum* and *M. hominis* urogenital infection

A total of 5164 of infertile patients (1554 women and 3610 men) were enrolled in the study. The overall prevalence of urogenital *C. trachomatis*, *U. urealyticum* and *M. hominis* detection in the total population was 5.3%, 22.8% and 7.4%, respectively (Table 1). Interestingly, *U. urealyticum* infection showed a remarkably higher overall prevalence than the others uropathogens analyzed. The prevalence of *C. trachomatis* infection was significantly higher in men than in women (5.8% versus 4.3%, $p=0.034$; Tables 1 and 2). Conversely, we found a significantly higher prevalence of both *U. urealyticum* and *M. hominis* infection in women (31.1 and 12.1, respectively) than in men (19.2 and 5.3%, respectively) ($p<0.001$ and $p<0.001$; Tables 1 and 2).

Table 1. Prevalence of urogenital infections in infertile patients

	Total (n=5164)	Women (n=1554)	Men (n=3610)
<i>Chlamydia trachomatis</i> , n (%)	275 (5.3%)	67 (4.3%)	208 (5.8%)
<i>Ureaplasma urealyticum</i> , n (%)	1176 (22.8%)	484 (31.1%)	692 (19.2%)
<i>Mycoplasma hominis</i> , n (%)	380 (7.4%)	88 (12.1%)	192 (5.3%)

Table 2. Prevalence of infections in infertile patients according to sex and age

Variables	Patients		<i>C. trachomatis</i> infection				<i>U. urealyticum</i> infection				<i>M. hominis</i> infection			
	n	n (prevalence)	Odds ratio	95% CI	p	n (prevalence)	Odds ratio	95% CI	p	n (prevalence)	Odds ratio	95% CI	p	
Sex														
Women	1554	67 (4.3%)	1.00 (ref.)			484 (31.1%)	1.00 (ref.)			188 (12.1%)	1.00 (ref.)			
Men	3610	208 (5.8%)	1.36	1.02-1.80	0.034*	692 (19.2%)	0.52	0.46-0.60	<0.001*	192 (5.3%)	0.41	0.33-0.50		
Age (y.o.)														
<i>Women</i>														
> 40	355	17 (4.8%)	1.00 (ref.)			107 (30.1%)	1.00 (ref.)			43 (12.1%)	1.00 (ref.)			
40 - 25	1130	47 (4.2%)	0.94	0.50-1.78	0.847	341 (30.2%)	0.88	0.66-1.17	0.372					
< 25	69	3 (4.3%)	0.96	0.26-3.50	0.949	36 (52.2%)	2.27	1.33-3.89	0.003*					
<i>Men</i>														
> 40	1356	88 (6.5%)	1.00 (ref.)			233 (17.2%)	1.00 (ref.)			72 (5.3)				
40 - 25	2161	106 (4.9%)	0.71	0.52-0.95	0.021*	433 (20.0%)	1.26	1.04-1.51	0.016*	116 (5.4%)	1.03	0.75-1.41	0.863	
< 25	93	14 (15.1%)	2.51	1.40-4.48	0.002*	26 (28.0%)	1.66	1.04-2.66	0.034*	4 (4.3%)	0.72	0.26-2.02	0.532	

Univariate analysis. 95%CI: 95% confident interval. A * $p < 0.05$ was considered statistically significant.

Associations among *C. trachomatis*, *U. urealyticum* and *M. hominis* urogenital infections

When assessing the co-infection between *C. trachomatis* and *U. urealyticum*, no significant association was found in either infertile women (OR: 1.62, 95% CI: 0.99-2.69, $p = 0.05$) or men (OR: 1.07, 95% CI: 0.76-1.52, $p = 0.05$) (Figure 1a, d). In fact, a similar prevalence of *U. urealyticum* was found in *C. trachomatis* infected (41.8%, 28/67) and *C. trachomatis* non-infected (30.7%, 456/1487) women (Figure 1a, Table 3). In addition, a comparable prevalence of *U. urealyticum* were found in *C. trachomatis* infected (20.2%, 42/208) and *C. trachomatis* non-infected (19.1%, 650/3402) men (Figure 1d, Table 3).

Table 3. *C. trachomatis*, *U. urealyticum* and *M. hominis* co-infections in infertile patients

	<i>C. trachomatis</i>				<i>M. hominis</i>			
	Women		Men		Women		Men	
	Positive (n=67)	Negative (n=1487)	Positive (n=208)	Negative (n=3402)	Positive (n=188)	Negative (n=1366)	Positive (n=192)	Negative (n=3418)
<i>U. urealyticum</i>								
Positive n (%)	28 (41.8%)	456 (30.7%)	42 (20.2%)	650 (19.1%)	171 (90.9%)*	313 (22.9%)	178 (92.7%)*	514 (15.0%)
Negative n (%)	39 (58.2%)	1031 (69.3%)	166 (79.8%)	2752 (80.9%)	17 (9.1%)	1053 (77.1%)	14 (7.3%)	2904 (85.0%)
<i>M. hominis</i>								
Positive n (%)	12 (17.9%)	176 (11.8%)	14 (6.7%)	178 (5.2%)	-	-	-	-
Negative n (%)	55 (82.1%)	1311 (88.2%)	194 (93.3%)	3224 (94.8%)	-	-	-	-

Univariate analysis. 95%CI: 95% confident interval. A * $p < 0.05$ was considered statistically significant.

Likewise, there was no significant association between *C. trachomatis* and *M. hominis* infections in either women (OR: 1.63, 95% CI: 0.83-3.07, $p = 0.05$) or men (OR: 1.31, 95% CI: 0.75-32.24, $p = 0.05$) (Figure 1b, e). From the 67 women infected with *C. trachomatis*, 12 were positive for *M. hominis* (17.9%), whereas 176 out of the 1487 *C. trachomatis*-negative women were positive for *M. hominis* (11.8%) (Figure 1b, Table 3). Also, a comparable prevalence of *M. hominis* was found in either *C. trachomatis* infected (6.7%, 14/208) or *C. trachomatis* non-infected (5.2%, 178/3402) men (Figure 1e, Table 3).

Interestingly, a significant association between *M. hominis* and *U. urealyticum* infection was found in women (OR: 33.84, 95% CI: 20.25-56.86, $p < 0.0001$) as well as in men (OR: 71.83, 95% CI: 41.89-187.10, $p < 0.0001$) (Figure 1c, Table 3). As detailed in Table 3, from the 188 women positive for *M. hominis*, 171 (91.0%) were positive for *U. urealyticum*. Similarly, a significantly increased prevalence of *U. urealyticum* (92.7%) was found in *M. hominis* positive men (Figure 1f). In fact, from the 192 men positive for *M. hominis* detection, 178 (92.7%) were positive for *U. urealyticum* (Table 3). Multivariate regression analysis further confirmed these tight associations, indicating that *U. urealyticum* and *M. hominis* act as mutual risk factors of infection either in women or men (Supplementary Table S1).

Noteworthy, only 2.0% of infected women (11 out of 539) and 1.5% of infected men (13 out of 871) were co-infected with the three uropathogens analyzed (Supplementary Figure S1).

Demographic parameters associated to C. trachomatis, U. urealyticum and M. hominis infection

Remarkably, *C. trachomatis*, *U. urealyticum* and *M. hominis* infections were significantly associated with patient sex and age (Table 2). In fact, univariate regression analysis revealed that *C. trachomatis* infection was more likely to be detected in infertile men than in women with an odds ratio of 1.36 (95% CI: 1.02-1.80, $p=0.034$) (Table 2). Moreover, a significant association was particularly found between *C. trachomatis* infection and men younger than 25 years (OR: 2.51, 95% CI: 1.40-4.48, $p=0.002$, Table 2) indicating that men, and especially those younger than 25 years, are at higher risk of infection than women (Table 2). Multivariate analysis further confirmed these associations (Supplementary Table S1).

On the contrary, *U. urealyticum* and *M. hominis* infections were associated to females, since men were less likely at risk of *U. urealyticum* and *M. hominis* infection than women with odds ratios of 0.52 (95% IC: 0.46-0.60, $p<0.001$, Table 2) and 0.41 (95% CI: 0.33-0.50, $p<0.001$, Table 2), respectively. In addition, when analyzing the association of infection with patient sex and age, it was found that *U. urealyticum* was more prevalent in patients younger than 25 years, either in women or men (OR: 2.27, 95% CI: 1.33-3.89, $p=0.003$, and OR: 1.66, 95% CI: 1.04-2.66, $p=0.034$, respectively; Table 2). Multivariate analysis further confirmed these data (Supplementary Table S1).

These results indicate that *C. trachomatis*, *U. urealyticum* and *M. hominis* urogenital infections are associated with patient sex and age. In fact, individuals younger than 25 years at the highest risk of *C. trachomatis* and *U. urealyticum* infection. Moreover, our data show that men are at higher risk of *C. trachomatis* infection and, conversely, women are at higher risk of *U. urealyticum* and *M. hominis* infection.

Discussion

Chlamydia trachomatis, *U. urealyticum* and *M. hominis* are amongst the most frequent sexually transmitted bacterial infections. Moreover, they have been associated to infertility in either females or males^{1-3,10,22}. Therefore, compelling data about the prevalence of urogenital *C. trachomatis*, *U. urealyticum* and *M. hominis* infection and their possible associations in infertile patients is of utmost importance. It has been shown that the prevalence of urogenital *C. trachomatis* infection in infertile men and women varies considerable across nations and regions and according to the subject population under study^{24,25}. Besides, although a growing number of studies about the prevalence of urogenital *U. urealyticum* and *M. hominis* infections in infertile patients have been reported during the last decade, compelling available data from large cross-sectional studies of is still scarce²¹⁻²³. Thus, we herein conducted a large observational investigation into urogenital *C. trachomatis*, *U. urealyticum* and *M. hominis* infections in women and men with infertility. Our data revealed a high overall prevalence of *C. trachomatis*, *U. urealyticum* and *M. hominis* infection in infertile patients. When analyzing data according to patient sex, the prevalence of *C. trachomatis*, *U. urealyticum* and *M. hominis* infection was 4.3%, 31.1% and 12.1% in infertile women, and 5.8%, 19.2% and 5.3% in infertile men, respectively. Univariate and multivariate regression analyses revealed that *C. trachomatis* infection was significantly more prevalent in infertile men whereas *U. urealyticum* and *M. hominis* infections were significantly more prevalent in infertile women. The higher prevalence of *C. trachomatis* urogenital infection we found in men with respect to women could be due to the fact that urogenital infections in men are much more frequently asymptomatic than in women⁸. Besides, our results are in line with recently reported data showing that the prevalence of *U. urealyticum* and *M. hominis* urogenital infections is higher in infertile women than in infertile men¹⁷. Furthermore, our results also showed that *C. trachomatis* and *U. urealyticum* infections were significantly more prevalent in young patients, particularly in those younger than 25, indicating that age as a risk factor and in agreement with previously reported data^{26,27}. In fact, it is known that sexually transmitted infections are directly related to sexual experience, having young people more frequent sexual intercourses, less consistency of condom use and one or multiple sexual partners^{26,27}. On the other hand, when assessing co-infections, only 2.0% of women and 1.5% of men were co-infected with the three uropathogens analyzed.

Our results support previously reported data. In a large observational study, Chen *et al.* found a prevalence of *C. trachomatis* infection of 3.5% in a population of 666 women seeking care for assisted reproduction in China, being significantly highest in patients younger than 25 years²⁸. Moreover, Piscopo *et al.* reported a prevalence of *C. trachomatis* infection of 3.7% in women with tubal infertility in Brazil²⁹. Besides and similar to our data, a prevalence of *C. trachomatis* infection of 4.3% in infertile men from Jordan has been reported³⁰. Moreover, our results are in line with reported data by Sleha *et al.*, who found a prevalence of *U. urealyticum* and *M. hominis* urogenital infection of 39.6% and 8.1%, respectively, in Czech women undergoing an initial infertility evaluation³¹. In addition, Gdoura *et al.* found a prevalence of *U. urealyticum* and *M. hominis* of 15.4% and 9.6% in male partners from infertile couples from Tunisia³². Similar data were also reported in infertile men from China³³.

However, significantly different prevalence rates of urogenital *C. trachomatis*, *U. urealyticum* or *M. hominis* infection in infertile women or men have been reported in other studies. In comparison with our data, a much lower prevalence of *U. urealyticum* infection was described in infertile men from Italy³⁴. Similarly, Boeri *et al.* recently reported lower prevalences of *C. trachomatis* and *M. hominis* infection in Italian men with primary infertility³⁵. Moreover, in a study conducted in infertile men from Spain, Veiga *et al.* found lower rates of *C. trachomatis*, *U. urealyticum* and *M. hominis* infection than ours (0.9%, 15.1% and 0.9%, respectively)⁶. In addition, considerably lower prevalence rates of *C. trachomatis*, *U. urealyticum* and *M. hominis* infection were reported in infertile women from the USA and Iran^{36,37}. Several factors could underlie these differences such as disparities in population under study (geographical location, age, ethnicity, religion, socio-economic status, access to medical care, etc.) or study design (prospective versus retrospective, patient population size, methods used for diagnosis, etc.). However, our results are in line with cumulative reported data and supported by the large patient population analyzed, including 5164 infertile individuals (1554 women and 3610 men).

Besides, our data revealed that *C. trachomatis* infection was not associated to either *U. urealyticum* or *M. hominis* in infertile women as well as in men. Conversely, and supporting recently reported data³⁸⁻⁴⁰, *U. urealyticum* or *M. hominis* were significantly associated to each other in either infertile women or men, indicating that *U. urealyticum* or *M. hominis* urogenital infection increased the risk of *M. hominis* or *U. urealyticum* co-infection. This tight infection association could be due to shared infection routes and/or pathophysiologic mechanisms^{1,10}.

To our knowledge, this is the first cross-sectional study to investigate the prevalence and association of *C. trachomatis*, *U. urealyticum* and *M. hominis* in infertile women and men from Argentina. Being one of the few cross-sectional study performed in Latin American countries^{21,23} and the large number of patients analyzed are the main strengths of our study. However, our study has some limitations such as it did not include control groups of fertile women and men, in whom the prevalence of the infections under analysis could have been different from their infertile counterparts. Moreover, we did not have the information of the partner couple of every infertile women or men enrolled, which could have provided important information about infection concordance.

In conclusion, our results indicate that urogenital *C. trachomatis*, *U. urealyticum* and *M. hominis* infections are prevalent in infertile patients. *C. trachomatis* infection was significantly more prevalent in infertile men whereas *U. urealyticum* and *M. hominis* infections were more prevalent in infertile women. Of clinical importance, *C. trachomatis* and *U. urealyticum* infections were more prevalent in young patients, especially in those younger than 25 years. Moreover, *U. urealyticum* and *M. hominis* showed to be reciprocal risk factors of their co-infection in either infertile women or men. Overall, these results point out the importance to include the microbiological screening of urogenital infections in the diagnostic workup of infertility. Moreover, they highlight the need to reinforce preventive strategies at the primary healthcare level. Increasing awareness among people and health care practitioners are efficient approaches for the prevention of infection transmission. Future research is needed to unveil the true impacts of these uropathogens and the underlying pathophysiological mechanisms, which would allow the identification of proper and efficient treatments to more effectively reduce the burden of infertility.

Methods

Study design, patients and samples

This prospective, cross-sectional study was performed on a total cohort of 5164 patients (1554 females and 3610 males) seeking care for infertility at a joint Academic, Urology and Reproduction Health Center from Cordoba, Argentina, between January 2015 and November 2019. Inclusion criteria were female or male aged 18-60 years, not taking antibiotics when sampling or during the last 3 weeks, and with a recent diagnosis of infertility based on a thorough self-reported sexual and medical history⁴¹. Vaginal-swab and semen samples were collected from female and male patients, respectively. Vaginal swabs were collected by a dedicated operator who inserted Dacron swabs into the vagina up to the vault and rotated the swab in the vaginal vault. The specimens were immediately transferred to a tube containing cervical cell preservation solution and processed for analysis. Semen samples were collected by masturbation after 2-7 days of sexual abstinence and processed within 1 h of collection.

Ethical approval

The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) standards and the Argentinian legislation for protection of personal data (Law 25326). The experimental protocol was approved by the Institutional Ethics Committee from the Hospital Nacional de Clinicas, Universidad Nacional de Cordoba (RePIS #3512). All patients provided a signed written informed consent form agreeing to share their own anonymous information prior to enrollment.

DNA extraction and C. trachomatis detection

Total DNA was extracted from vaginal or semen samples and *C. trachomatis* infection detected by polymerase chain reaction (PCR) using the *Chlamydia trachomatis* 330/740 IC PCR kit (Sacace Biotechnologies Srl, Como, Italy) and within 4 h of sample collection.

M. hominis and U. urealyticum detection

M. hominis or *U. urealyticum* infections were assessed by culture using the commercially available colorimetric kit Mycofast RevolutioN (ELITech Group, Puteaux, France). Briefly, 100 µl of vaginal swab seeded transport medium or semen were added to the *M. hominis* and *U. urealyticum* wells, sealed and incubated at 37°C ± 1°C for 24 h. After incubation, orange or red color changes indicated the presence of *M. hominis* and/or *U. urealyticum*, respectively.

Statistics

Demographic characteristics (age, sex) and co-infections were compared between infected and non-infected patients using the chi-square test and the odds ratios (OR) and 95% confidence intervals (CI) were calculated. Univariate and multivariate logistic regression analyses for all variables were performed to determine associations or risk factors for infections. The SPSS statistical software package (version 17.0; SPSS Inc., Chicago, USA) and GraphPad Prism 8.0.1 were used for analyses. A $p < 0.05$ was considered statistically significant.

Declarations

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Authors' contributions

R.D.M., G.M., and R.M. participated in the conception and design of the study. G.M., D.A.P., A.D.T., C.O. and R.I.M. participated in the acquisition, analysis and interpretation of data. R.D.M. wrote the manuscript text and D.A.P. prepared the figure and tables. All authors critically reviewed the manuscript and approved the final version before submission.

Additional Information

Competing interests

The authors declare no competing interests.

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Figures

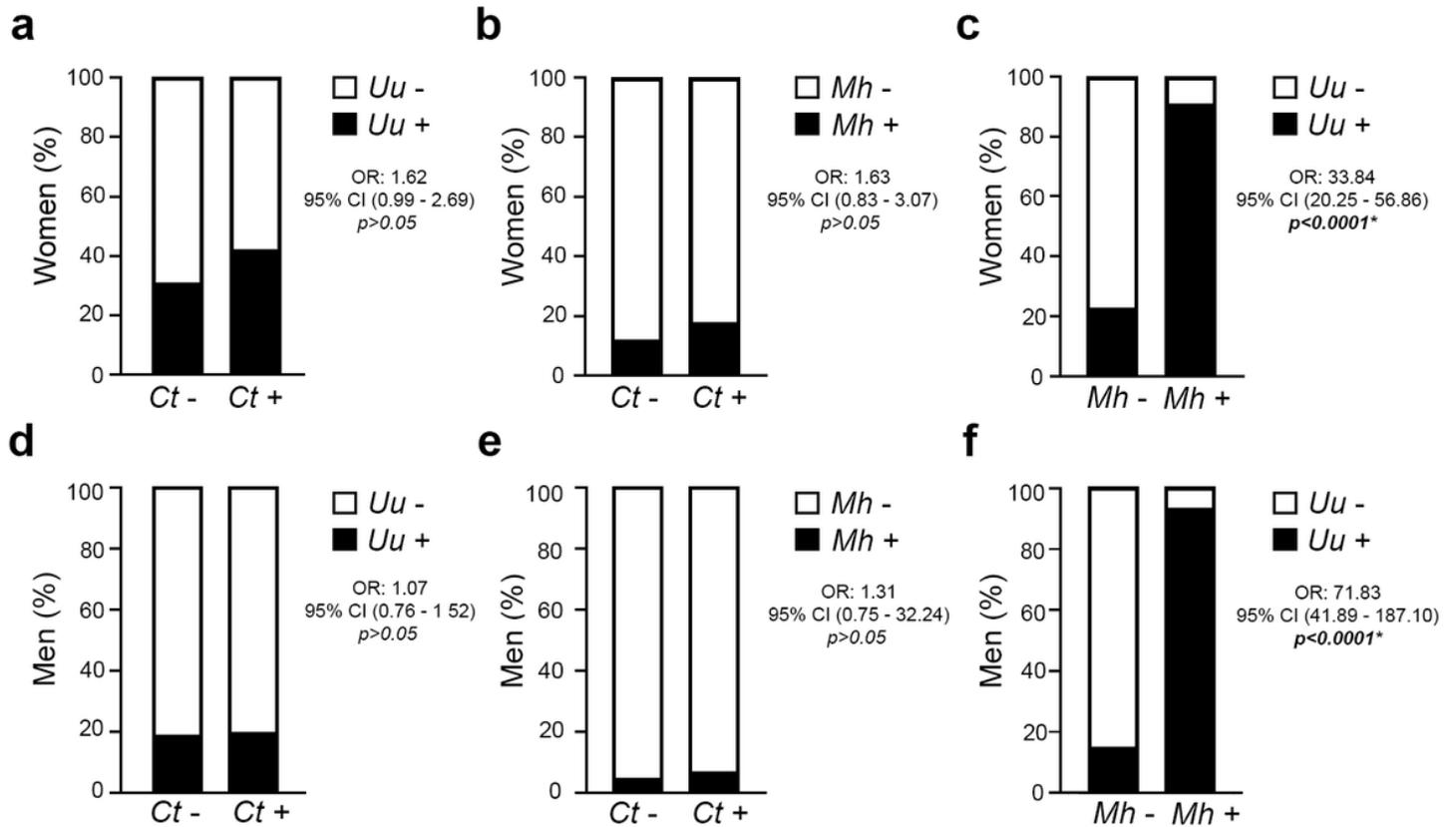


Figure 1

C. trachomatis (Ct), U. urealyticum (Uu) and M. hominis (Mh) co-infections/colonizations in infertile patients. Frequency of positive Uu infection/colonization in Ct-infected (Ct+) or Ct-non infected (Ct-) women (a) or men (d). Frequency of positive Mh infection/colonization in Ct-infected (Ct+) or Ct-non infected (Ct-) women (b) or men (e). Frequency of positive Uu infection/colonization in Mh-infected/colonized (Mh+) or Mh-non infected/colonized (Mh-) women (c) or men (f). Data are shown as frequency. Chi square test were assessed and odds ratio with 95% confident interval (OR, 95% CI) calculated. A * $p < 0.05$ was considered statistically significant.

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