

# *Prevalence of two species of genital mycoplasmas among infertile women attended to infertility clinic in Thi-Qar*

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## **Abstract**

The aim of this study was to find the incidence of urogenital mycoplasma such as *Mycoplasma hominis* and *Ureaplasma urealyticum* among women attending infertility clinic. The total number of (n = 300) endocervical swab samples taken from women attended to Maternity Hospital outpatient clinic in Thi-Qar. One hundred fifty swabs was taken from women has a period of infertility of at least one year and both partners had ready the basic infertility investigations (group I). One hundred fifty swabs were taken from fertile women who attended the family planning clinic Thi-Qar (group II). It was found that the incidence of *Ureaplasma urealyticum* was significantly higher in the infertile (22%) than

in the fertile group (4.7 %) ( $Z = 5.6, P < 0.01$ ). The incidence of *Mycoplasma hominis* was not significantly different between the infertile (26.6 %) and the fertile (21.3 %) groups had been studied ( $Z = 1.71, P < 0.05$ ). During the period of the present study, the infertile women with positive culture for *Ureaplasma urealyticum* (33 women) and their husbands were treated with doxycycline ± azithromycin oral capsules that successfully eradicated the *Ureaplasma urealyticum* from the endocervix of the infertile women. The pregnancy rate after eradication of *Ureaplasma urealyticum* was (28.5 %) in couples with unexplained infertility which was higher than in couples with explained infertility (6.6 %).

## Introduction

Annually, there are an estimated 500 million new cases of sexually transmitted infections (STIs) which are acquired, worldwide according to (WHO)<sup>1</sup>. If diagnosed in time, these infections can be treated easily with minimal morbidity as well as decreased economic burden<sup>2</sup>. *Mycoplasma genitalium*, *Mycoplasma hominis* and *Ureaplasma* spp (collectively, genital mycoplasmas) are most often implicated in genital or reproductive health conditions<sup>3</sup>. *Ureaplasma urealyticum* and *Mycoplasma hominis* can be found in the cervix or vagina of sexually mature women<sup>4-8</sup>. Mycoplasmatales are associated with infection of the

genitourinary tract, , neonatal morbidity and mortality, and reproductive failure. Infection with genital mycoplasmas has been linked with infertility<sup>9-11</sup>. *Ureaplasma* spp. are the main cause of nonchlamydial, nongonococcal urethritis and acute prostatitis<sup>12</sup>. *U. urealyticum* in a large proportion of healthy women complicates the assessment of the pathogenic roles of this organism, but several studies have indicated that genital colonization of the *U. urealyticum* can be associated with an increased risk of developing certain pathogenic conditions<sup>13-15</sup>. During the past decade, evidence has accumulated of causative role of *U. urealyticum* in human infertility. *U. urealyticum* was detected at a higher frequency in infertile women<sup>16, 17</sup>.

The aim of this study is to detect *M. hominis* and *U. urealyticum* in genital tract specimens from vaginal swabs obtained from infertile female patients.

## Patients and methods

### I- Study population:

From the first of July 2013 till the first of October 2014 (period of 15 months), endocervical swabs were taken from 300 women and studied for the presence of genital mycoplasma (*Uu.* and *Mh*). They were divided into two groups: Group I: 150 women were selected from infertility clinic. They all shared the following criteria: (1) A period of infertility of at least one year. (2) Both partners had ready the basic infertility investigations. After taking full history & clinical examination, their

investigations were studied and they were further subdivided into two subgroups of: A – Explained infertility (116 women, 77.4 %) B – Unexplained infertility (34 women, 22.6 %). Group II: Were 150 fertile women who attended the family planning clinic. Any patient who had received antibiotic therapy for any reason during the preceding two weeks was excluded from the study. All women agreed to participate in the study by verbal consent.

## 2- Collection of specimens:

A freshly prepared transport medium was brought from the central laboratory to the Maternity Hospital outpatient clinic for both infertility and family planning in Thi-Qar. The woman was put in the lithotomy position, ausco speculum was introduced into the vagina, the cervix was carefully cleaned with a sterile cloth, cervical mucus was taken with a sterile cotton – tipped swab moistened in saline. The specimens had taken from the endocervix were put immediately in to Stuart transport medium and quickly taken back to the central laboratory,

as any delay of more than 1-2 hours between collections of mycoplasmas & its transport will affect the results<sup>19</sup>.

Swabs were kept in tubes containing transport medium specially to maintain the swabs wet<sup>18</sup>. Each specimen was encased in ice bag until be taken to the laboratory for bacteriological analysis .Specimens were first incubated at 370Cfor 30 minutes, and then a loop-full from each sample was transferred to Arginine broth and A7 broth for Mycoplasma hominis and Ureaplasma urealyticum respectively<sup>20</sup>.

## Statistical analysis:

The rate of positive genital mycoplasmas (Uu. and Mh.) infection was expressed in percentages. Z test was used to test difference among the groups and it was considered significant if the Z value more 1.96 and P-value less than 0.05.

## Results

At the conclusion of the study the data of 300 women (150 infertile and 150 fertile) have been collected and analyzed. The mean age of the infertile women in year = 32.03 + 7.08 while the mean age of the fertile women = 31.1 + 5.6, t-value = 1.261 NS .

(1)Prevalence of genital mycoplasmas (Uu. and Mh.) in infertile and fertile and women . Genital mycomplasmas (Uu. and Mh.) were detected I 112 (37.3 %) out of 300 women .

In the infertile group the incidence of genital mycoplasmas ( Uu. and Mh. ) was 73 ( 48.6 % ) out of 150 . 40 (26.6 %) were culture positive for Mh., while 33 (22% ) were culture positive for Uu. The remaining 77 (51.4%) were culture negative .

In the fertile groups , the rate of isolation of genital mycoplasmas ( Uu. and Mh. ) was 39 ( 26 % ) out of 150 , 32 ( 21.3 % ) Mh. while 7 (

4.7 % ) Uu. The remaining 111 (74 %) were culture negative .

From the previous data, It has been found that there was a statistically significant difference in the prevalence of genital mycoplasmas (Uu. and Mh.) in the infertile women (48.6%) and the fertile women (26%),  $Z= 5.75$  ,  $P<0.001$ , as shown in figure 1.

In order to evaluate whether this difference in the prevalence of genital mycoplasmas (Uu. and Mh.) between the infertile and the women was due to Mh. or Uu. or both a comparison was made for each microorganism alone between the infertile and the fertile groups .

In case of Uu. the incidence in the infertile women was (22%) as compared to the fertile control (4.7 %). The difference between the two percentages was statistically significant ( $Z = 5.6$ ,  $P < 0.001$ ).

While there was no statistically significant difference in the isolation of Mh. between the infertile females (26.6 %) and the fertile females (21.3 %), ( $Z = 1.71$ ,  $P > 0.05$ ) as illustrated in figure 2.

(2) The frequency of Uu. in cervical mucus of the infertile women according to the cause of infertility :

-In females with explained infertility 24 (20.6 %) out of 116 have been shown to harbor Uu as compared to seven (4.7 %) out of 150 fertile female. The difference was statistically significant between the two groups ( $Z = 4.25$ ,  $P > 0.001$ ).

-Those women with unexplained infertility 9 (26.4 %) out of 34 were culture positive for Uu. as compared to 7 (4.7 %) out of 150 fertile control the difference between two percentages was statistically significant ( $Z= 4.2$ ,  $P < 0.011$ ).

-Although the percentage of infected female who had unexplained infertility ( 26.4 % ) was higher than those who had explained infertility ( 20.6 % ) but the difference between the two percentage was not statistically significant (  $Z = 0.63$  ,  $P 0.05$  ) , as shown in Figure 3.

(3) Prevalence of Uu in the infertile women according to the duration of infertility :

It was found that the most affected females were those with the duration of infertility of 6 – 10 years, but no pattern of an increase in the percentage of their having Uu infection was observed as the duration of infertility was prolonged as demonstrated in table (1).

(4)Frequency of Uu infection in the infertile women according to their clinical features at presentation :

It has been found that (48.5 %) of the infected infertile women were asymptomatic which was higher than percentages of the infected infertile women with other presentations, as illustrated in table (2).

**Figure 1. Demonstration the prevalence of positive genital mycoplasmas (Uu. and Mh.) in the end cervix of the infertile and fertile women.**

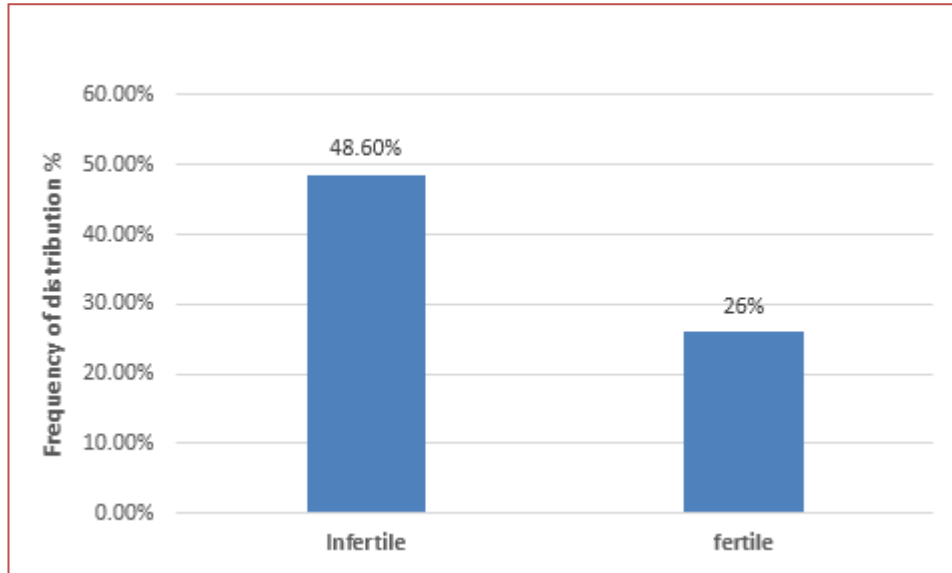


Figure 2. A bar chart demonstrates the frequency distribution of each and Mh. in the infertile and fertile groups.

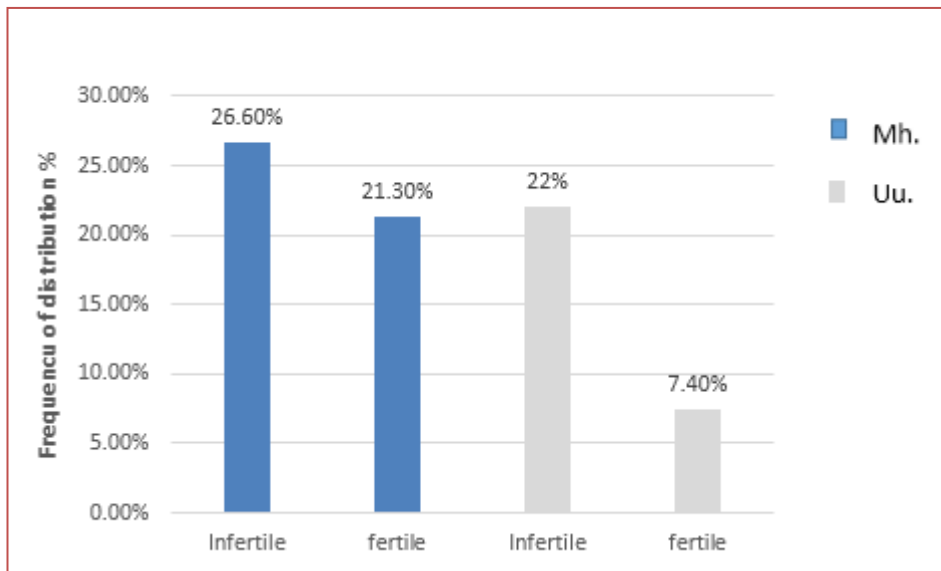
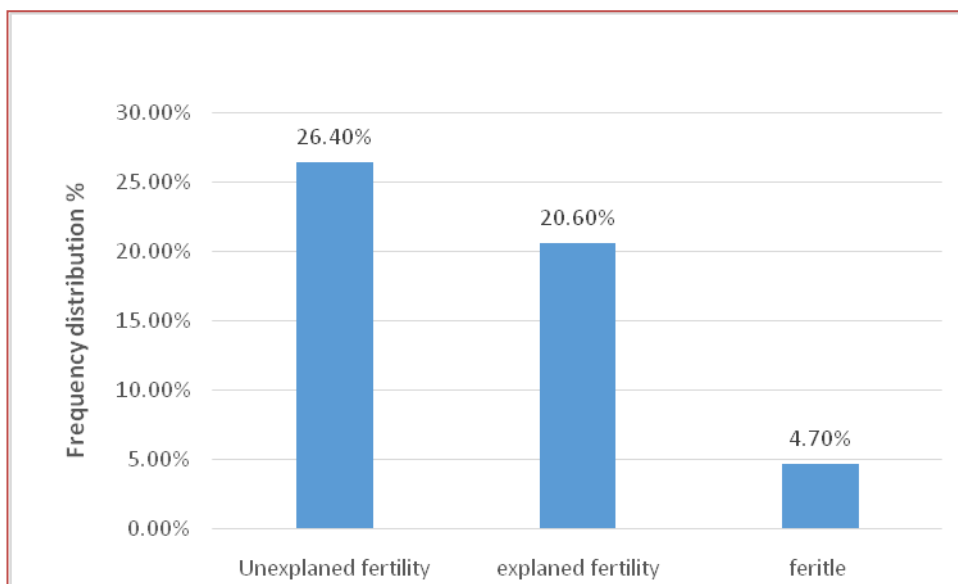


Figure 3. Shows the frequency distribution of Uu. infection in the infertile women according to the cause of infertility.



**Table 1. Shows the frequency of isolation of Uu in the infertile women according to the duration of infertility.**

Duration of infertility	N = 33	%
1 – 5	10	30.3
6 – 10	15	45.5
11 – 15	5	15.15
16 – 20	3	9.09

N = Total number of the infertile women with positive end cervical culture for Uu.

**Table 2. illustrates the prevalence of Uu in the endocervix of the infertile women according to their presentations.**

Clinical features at presentation	N = 39	%
Asymptomatic	16	48.5
Urinary symptoms	7	21.2
Vaginal discharge	6	18.18
Itching	5	15.15
Abdominal pain	5	15.15

N = total number of the infertile women with positive Uu. culture.

\* Some patients report more than one symptom.

#### Second Part of the study (Treatment trial)

The 33 infertile couples (explained and unexplained infertility) with positive endocervical Uu. cultures were treated with doxycycline oral capsules of 100 mg / day for 10

days from the first day of the cycle , and the couples were advised to use condom during the period of treatment .

After completion of the first course of treatment, the endocervical swabs were repeated. A course of treatment was used with azithromycin 500 mg on day 1 followed by 250 mg daily for 4 days if treatment fails( whenever the repeated endocervical swabs were still positive).Six couples were excluded from the study because of failure of follow up.

The remaining 27 couples, 22 (81.5 %) of them became culture negative, while five

(18.5%) were still culture positive for Uu .Those 22 patients who became culture negative, 15 of them had explained infertility and seven had unexplained infertility .

Of the seven couples who had unexplained infertility, two women (28.5 %) became pregnant.Out of the 15 couples with explained infertility, one woman (6.6 %) got pregnant.

## Discussion

It can be argued that the presence of any microorganism in the upper female reproductive tract, either acute or chronic can adversely affect fertilization, implantation and capacity to keep the embryo because of the inflammatory reactions generated by the presence of these microorganisms<sup>22</sup>.

Mycoplasmas constitute a unique and enigmatic group of microorganisms; however, Dienes and Edsa, who recovered such organisms from a Bartholinian abscess, reported the earliest isolation of mycoplasmas from a human source. Since then, sporadic reports have implicate mycoplasmas in diseases of the female genitalia and in complications of pregnancy. Such infections might not give rise to symptoms until and unless, infertility or reproductive wastage was to occur<sup>23-25</sup>.

The mycoplasmas role in the genital and extra genital systems is depends on epidemiologic data<sup>22</sup>. Although the implication of ureaplasmas in lower urogenitaltract infections and their

adverse effect on pregnancy seem to be well established, their role in the upper genitourinary tractinfection is not so obvious<sup>26</sup>.There are many reports linking ureaplasmas with infertility. Indeed, genital ureaplasmas are isolated more commonly from infertile subjects than from healthy controls<sup>27-29</sup>. The association of genital ureaplasmas with a variety of pathological conditions in women is based on the detection of these potential pathogens in the specimens from the lower genitourinary tract e.g., vaginal or endocervicalswabs. However, there is little if any evidence documenting the detection of ureaplasmas in the sterile samples from the upper genitourinary tract of asymptomatic women.Colonization of the vagina or cervix with ureaplasmas can lead to the ascending infection of the upper genital tract with subsequent inflammation of these structures. Longlastinginflammation within upper genital tract can cause scarring of the inflamed tissue and can contribute to the development of reproductive system disorders such asinfertility<sup>27</sup>.

The distribution of agents and the susceptibility of the antibiotics changed in terms of the time and geographical region, Azithromycin was more effective than doxycycline in treating patients infected with *M genitalium*. The extended course of azithromycin was highly effective<sup>(30)</sup>. A number of different antibiotics have been used to treat *M genitalium* infection. In a preliminary open study from Scandinavia a trend towards improved outcome with longer duration of therapy was observed— azithromycin 1 g immediately eradicated 85% (11/13) of the *M genitalium* infections whereas a dose of 500 mg on day 1 followed by 250 mg daily for 4 days eradicated 95% (19/20) of infections. With varying degrees of success. Tetracyclines initially looked promising but more recent studies suggest that failure to fully eradicate the infection occurs in a high proportion of cases treated with these agents<sup>(30)(31)(32)</sup>. In our study agree with the results of previous studies above. Also we found treatment of genital mycoplasmas helped in achieving a pregnancy .



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