**Frequency of Anti-**Mycoplasma hominis** Antibodies in Infertile Women Referred to Al-Zahra Hospital in Tabriz, Iran**

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**ABSTRACT**

**Background and Objectives:** Infertility is one of the important causes of anxiety in couples. Infections caused by genital Mycoplasmas may have harmful effects on the reproductive health of women, and sometimes lead to infertility. This study was designed to determine frequency of anti-**Mycoplasma hominis** antibodies in infertile women at Al-Zahra Hospital, Tabriz, Iran.

**Methods:** In this cross-sectional study, serum from 184 infertile women and 100 healthy pregnant women were tested for presence of **M. hominis** IgM and IgG antibodies by ELISA. Data collected were analyzed in SPSS (version 17) using t-test at significance level of 0.05.

**Results:** The frequency of anti-**M. hominis** IgG was significantly higher in infertile women compared to healthy controls. The frequency of anti-**M. hominis** IgM did not differ significantly between the infertile and control subjects. The majority of the women infected with the bacterium were in the 21-30 and 31-40 years age range. There was no significant correlation between tubal factor infertility and seropositivity for **M. hominis** antibodies. Moreover, the dwelling of the participants had no relationship with the frequency of anti-**M. hominis** antibodies.

**Conclusion:** The frequency of infertile women infected with this bacterium is high in Tabriz. Therefore, it is important to perform microbial screening for this bacterium in infertile couples.

**Keywords:** Mycoplasma hominis, Infertility, Women, Tabriz.

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INTRODUCTION
Infertility is one of the most important causes of anxiety in couples, which affects their personality traits (1). It is defined as the inability to conceive after regular unprotected intercourse for a year, or carry a child to live birth (2, 3). Epidemiologic studies have indicated that 80% of normal non-contracepting women become pregnant within one year. Infertility is divided into primary infertility (no history of a previous pregnancy) and secondary infertility (pregnancy has occurred before) (4). A survey by the World Health Organization (WHO) has shown that 43% of women and 30.1% of men suffer from infertility worldwide (5). There are several factors involved in infertility, including environmental and genetic factors and infections (6, 7). There is a significant relationship between subclinical infections and fertility. Infection may cause infertility through different mechanisms. *Mycoplasma hominis* is the causative agent for reproductive tract infections (8). These infections are often asymptomatic and have negative effects on the reproductive health (9). *Mycoplasma* is a part of the normal flora of the genitourinary tract but can act as an opportunistic pathogen (10). Tubal factor infertility (TFI) is one of the most important causes of infertility in women. Uterine tube infections cause severe pain and irritation, and can lead to infertility or ectopic pregnancy. Most of these infections are caused by *Mycoplasma*, *Ureaplasma* species and *Chlamydia trachomatis* (11-13). *M. hominis* prevents implantation by producing neuraminidase, causing ovule poisoning. In addition, the microorganism can cause abortion or decrease the number and efficiency of sperms by altering pH of vaginal area. If not diagnosed and treated, these infections could become chronic and cause pelvic inflammatory disease and infertility. Clinical diagnosis of the bacteria and screening of young infertile couples are necessary (9). Limited number of studies has been performed on diagnosis of *M. hominis* and its effects on infertility in Iran. For instance, Najarpiraye et al. found a significant relationship between *M. hominis* and cervicitis (14). In addition, Salari et al. reported a significant difference in the prevalence of *M. hominis* between infertile women and healthy controls (15). However, limited information is available on the effects of *M. hominis* on infertility in Tabriz, Iran. Therefore, this study aimed to detect and determine the frequency of anti-*M. hominis* antibodies in serum samples of infertile women.

MATERIAL AND METHODS
In this cross-sectional study, plasma samples were randomly selected from infertile women referred to Al-Zahra Hospital in Tabriz between November 2014 and April 2015. The study has been approved by the ethics committee of the Department of Sciences and Technology of Tabriz University of Medical Sciences (Code: TBZMED.REC.1394.1211). Sample size included 184 infertile women and 100 healthy pregnant women (as controls) aged 16-45 years. The women with history of chronic diseases like tuberculosis and immunologic disorders were excluded from the study. The participants completed a questionnaire on information including dwelling, age, history of infertility in relatives and previous proceeding. In order to detect anti-*M. hominis* IgG and IgM antibodies, 5-ml blood samples were collected in capped vacuum tubes under sterile condition. The tubes were centrifuged at 2000 rpm for 10 minutes. Serum was separated and transferred into a microtube and kept at -70 °C. *M. hominis* IgM/IgG ELISA Kits (Vircell Co., Germany) with 98% sensitivity and 97% specificity and an ELISA Plate Reader (Awareness 14; Model 3200) were used for analysis of the samples. The concentration of antibodies obtained were calculated and compared with standard values. OD of antibodies was calculated according to the following formula: Antibody index = (sample OD/ cut-off serum mean OD) x 10. The results were interpreted as follows: Negative: <9, ≥ 9 – <11: Equivocal, ≥11: Positive.

Data collected were analyzed in SPSS software (version 17) using t-test, Pearson correlation coefficient and one-way analysis of variance. P-values less than 0.05 were considered as statistically significant.

RESULTS
Of 284 women, 214 were from urban areas and 70 from rural areas. The participants were divided into quartiles based on their age: less than 20, 21-30, 31-40 and more than 40 years. The majority of the women were in the 21-30
There was no significant relationship between age and the amount of anti-\textit{M. hominis} IgG and IgM in infertile women (Table 2). Moreover, there was no significant relationship between the location of residence and frequency of anti-\textit{M. hominis} antibodies (Table 3).

Moreover, there was a significantly higher amount of anti-\textit{M. hominis} IgM antibody compared to the controls \((P=0.000)\). There was no significant difference between the two groups in terms of anti-\textit{M. hominis} IgG antibody level (Table 1). Moreover, there was no significant relationship between the dwelling and frequency of anti-\textit{M. hominis} antibodies (Table 3).

### Table 1 - Frequency distribution of IgG and IgM antibodies

| Antibody titer | IgG | IgM | p-value* |
|---------------|-----|-----|----------|
| <9 (Negative) | Fertile 88 (88) | Infertile 94 (51.08) | 90 (90) | 174 (94.56) | 0.000 | 0.159 |
| 2<9 to ≤11 (Equivocal) | 8 (8) | 22 (11.95) | 4 (4) | 8 (4.34) |
| ≥11 (Positive) | 4 (4) | 68 (36.95) | 6 (6) | 2 (1.08) |
| Total | 100 (100) | 184 (100) | 100 (100) | 184 (100) |

*Independent t-test and Levene's test for equality of variances. Data presented as n (%). \((n=284\) in each group)

### Table 2 - The number of subjects in each age group

| Pregnancy status | Age range (years) | Total |
|------------------|-------------------|-------|
| Fertile women    | <20 | 21-30 | 31-40 | >40 | 100 |
| Infertile women  | 8 | 50 | 36 | 6 | 184 |
| Total            | 28 | 132 | 104 | 20 | 284 |

*Pearson correlation coefficient was used for statistical analysis

### Table 3 - Frequency distribution of IgG and IgM antibodies according to the location of residence

| Antibody titer | IgG | IgM | Total |
|---------------|-----|-----|-------|
| <9 (Negative) | Urban 126 | Rural 56 | 182 | Urban 196 | Rural 68 | 264 |
| 2≥9 to <11 (Equivocal) | 24 | 6 | 30 | 12 | 0 | 12 |
| ≥11 (Positive) | 64 | 8 | 72 | 6 | 2 | 8 |
| Total | 214 | 70 | 284 | 214 | 70 | 284 |

*One-way ANOVA was used for statistical analysis

### Table 4 - Frequency distribution of TFI in the study

| Fertility explanation | Uterus tube | Total | P-value* | P tubal factor |
|-----------------------|-------------|-------|----------|----------------|
| Yes                   | Both opened | Both closed | 1 opened/ 1 closed | 100 | IgG 0.003 | IgM 0.074 | IgG 0.074 | IgM 0.729 |
| No                    | 164 | 12 | 8 | 184 | IgG 0.003 | IgM 0.074 | IgG 0.074 | IgM 0.729 |
| Total                 | 264 | 12 | 8 | 284 | | | | |

*One-way ANOVA was used for statistical analysis
DISCUSSION

Infertility is becoming a global health problem (16). Average rate of infertility is 8-12% in different countries. Infections can affect the fertility process through different mechanisms (17). M. hominis is a part of the normal genital flora of both men and women but may cause infertility. In this study, anti-M. hominis IgG antibody titer was significantly higher in infertile women compared to pregnant women. However, level of anti-M. hominis IgM antibody did not differ significantly between the two groups. These results are inconsistent with the results of Miron et al. in terms of anti-M. hominis IgG antibody titer (18), and in partial agreement with studies of Gunyeli et al., Grzesko et al., Reid et al. and Fenkci et al. in terms of the anti-M. hominis IgM antibody titer (19-22). We also found no significant association between dwelling and the frequency of anti-M. hominis. Moreover, there was no significant relationship between age of the subjects and frequency of anti-M. hominis antibodies. However, frequency of infection with the bacterium was higher in the women aged 21-30 and 31-40 years. These results are in line with the results of Ahmadi et al. (23) and Najarpirayeh (14).

Blockage of fallopian tubes had no significant relationship with the level of anti-M. hominis antibodies. This is inconsistent with the results of Baczynska et al. (24) but in agreement with the study of Costoya et al. (25). The difference in the results of previous studies may be due to differences in sample size, the diagnostic methods used and sociocultural conditions.

CONCLUSION

The frequency of anti-M. hominis antibodies is relatively high in infertile women. Considering the significant association between infertility and presence of M. hominis antibody, it is important to perform microbial screening for this bacterium in infertile couples.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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