

Original Article

***Mycoplasma* Infection in Pyospermic Infertile and Healthy Fertile Men**

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Abstract

Background: Infections are one of the correctable causes of infertility with low cost and cost effective treatment. The 50% of infertile cases is related to men in some way, and 30% of them are absolutely related to them. Mycoplasmas are the smallest microorganisms with capability of DNA replication. Present study is planned to compare the mycoplasma infection in infertile men and men with established fertility.

Materials and Methods: 45 Semen samples were collected from case and control persons who referred to Royan Infertility and Fertility Institute between 2004 and 2005 and stored in -20°C until time of test. DNA was extracted from semen using phenol chloroform. PCR reaction was done by *mycoplasma* specific primers.

Results: *Mycoplasma genitalium* gene was amplified in 6 (40%) cases from 15 infertile semen samples and 11 (36.6%) from 30 control semen samples.

Conclusion: Probability of genital infection, at least, in these studies group, is very lower than other communities' reports.

Keywords: *Mycoplasma*, pyospermic infertile men, fertile men

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Introduction

As well as attempts to control population and development of contraceptive methods by the WHO in the past decades, it is essential to discover and dissolve the infertility problems. Today, 13-18% of couples in the productive age are infertile all over the world¹. Thus, mental health's of these persons, which consist of considerable percentage of population, are at risk. Also infertility and in correct non-scientific and non-realistic encounter to it, resulted in

destruction of families as the community health columns.

Solutions for infertility problems not only associated with many risks, but also with high costs² and are not available for all levels and regions in the world, finally success percentage of these methods are not complete. Thus, attempts to find low cost methods are explained. Infections are one of the correctable causes of infertility with low cost and cost effective treatment. The 50% of infertile cases is related to men in some way, and 30% of them are absolutely related to them¹.

The 50% of infertile men are curable. So, researcher's concern are discover of infertility etiology in men, specially discover of correctable causes³⁻⁵.

Gram negative infections can results in occlusion of epididymis and cause to sperm motility impairments⁶. Also, *Chlamydia trachomatis* and *mycoplasma* infections are involved in infertility and many researches have done to establish the role of these micro organisms³⁻⁵.

Mycoplasmas are the smallest microorganisms with capability of DNA replication. These organisms should enter to the host cells because they don't have cell wall and their genome is very small so need some host enzymes to replication. In the other word, they are intracellular parasites. Their slow growing on media results in difficult replication in laboratory condition⁷.

Previous methods for detection of *mycoplasma*, which are involved in infertility, were: serological and microbe culture methods have no sufficient specificity and sensitivity. The PCR is the gold standard method for diagnosis of *mycoplasma* with high speed and accuracy.

Present study is planned to compare the *mycoplasma* infection in infertile men and men with established fertility.

Methods

This case control study was performed on Royan Infertility and Fertility Institute on 45 samples, in Tehran capital city of Iran, between 2004 To 2005. Semen samples were collected from 15 cases and 30 control persons and stored in -20°C until time of test.

Case group: This group consists of 15 men who were referred by physician to the Royan Infertility and Fertility Institute with complaint of infertility. Diagnosis process was performed in this method: first the women visited by the gynecological specialist and then with an administration of spermogram from their husbands, they were referred to the urologist who was record the identification information, history, physical exam and fertility laboratory tests results in the man's sheet. Characteristics of this group were, refer to Royan Institute with complaint of infertility, existence of pyospermia and rule out of other causes of infertility

in the couples in the primary investigations.

Control group: This group was selected among those men that: first fertility was established in them and no problem was seen in their semen analysis.

Meaning full difference between case and control groups can suggest the role of *mycoplasma* in infertility. Thus, control group were selected among men referring to Shahid Jafari Health Center in order to family management and after consultation, in the primary steps, candidate for vasectomy. Because of positive history of fertility in these men, their semen samples should be tested three times. This group was matched with the case group in regard of age.

DNA extraction and PCR amplification: DNA was extracted from semen using phenol chloroform as previously described⁸. PCR reaction was done by *Mycoplasma* specific primers. PCR reaction was done using: primers MgeniF 5'-CCT TAT CGT TAG TTA CAT TGT TTA A-3 and MgeniR 5'-TGA CAT GCG CTT CCA ATA AA -3' witch were amplified 340 bp of *Mycoplasma genitalium* genome. PCR reaction was included 2 micro liters of DNA (containing 100 ng), 150 μM dNTP, and 40 Picomoles each of forward and reverse primers, 1.5 mM MgCl₂, 1 X PCR buffer and 1.25 units of Taq DNA polymerase in 30 μL final volume. PCR amplification was performed by following parameters: denaturing at 94°C for 30 second, annealing at 62°C for 30 second and extension at 72°C for 30 second. These processes were repeated for 30 cycles. Reaction was settled at 94°C and 72°C for 5 minutes before and after PCR cycling, respectively.

Detection of PCR product: PCR product was electrophoresed on 2% agarose gel and DNA band was observed by UV Transilluminator after ethidium bromide staining.

Results

Mycoplasma genitalium gene was amplified in 6 (40%) cases from 15 infertile semen samples and 11 (36.6%) from 30 controls semen samples (Table 1). From these data, it's suggested that no considerable statistically significant difference between these two groups. For above table, odds ratio can be:

$$\text{Odds ratio} = \frac{6 \times 19}{9 \times 11} = 1.15.$$

Table 1: Results of *mycoplasma genitalium* PCR reaction for infertile and healthy men.

Result Sample type	Positive by PCR		Negative by PCR		Total
	Frequency	Percent	Frequency	Percent	
Infertile (case)	6	40	9	60	15
Fertile (control)	11	36.6	19	63.4	30
Total	17		28		45

Discussion

Mycoplasma is the smallest microorganisms with capability of DNA replication. These organisms should enter to the host cell because they don't have cell wall and their genome is very small (obligate intra cellular parasite). Their growth in culture media is very slow for laboratory detection and they needed a reliable diagnostic method in routine diagnostic laboratory⁷.

The rates of *Mycoplasma* species infection in men with non-gonococci urethritis with detected by culture in case and control groups were 19.2% and 7.2% for *Ureaplasma urealyticum*, 7.2% and 0.8% for *Mycoplasma genitalium*, and 2.4% and 1.6% for *Mycoplasma hominis* respectively⁹.

The rate of infection assayed by indirect hemagglutination in China for immoral persons was 25.47% for *Ureaplasma urealyticum*, 18.22% for *Chlamydia trachomatis* and 8.80% for *Mycoplasma hominis* that is higher than of infection in healthy controls¹⁰.

The prevalence of infection in patients with non-gonococcal urethritis in Japan was 47.7% for *Chlamydia trachomatis*, 17.0% for *Mycoplasma genitalium*, 16.3% for *Ureaplasma urealyticum* (biovar 2), 7.8% for *Ureaplasma parvum* (biovar 1) and 2.6% for *Mycoplasma hominis*¹¹. *Mycoplasma genitalium* infection was reported at 5.3% in men with symptomatic urethritis in Bristol¹² and 13.8%¹³ and 6.3%¹⁴ for Swedish women and 4.1%¹³ and 6%¹⁴ for Swedish men. *Chlamydia trachomatis* was reported at 4.7%¹³ and 4.5%¹⁴ for Swedish women and 9.1%¹³ and 5.4%¹⁴ for Swedish men.

If we concern that prevalence of disease caused by *Mycoplasma* species in our country is the same as those with other researches were done, it should be expected that in infertile men with positive test of *Mycoplasma*, 42% are infected with *mycoplasma* genus and 21% of control group are infected.

Correlation between pyospermia and infertility:

Because of selection of case group from infertile men with pyospermia, attention to the correlation of pyospermia and infection in urogenital tract and relation of them with infertility seems to be essential.

There are different reports from prevalence of pyospermia in infertile men. The prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* were reported as 7% and 20% respectively in patients with sterile pyuria in Austria¹⁵. Its prevalence is reported from 6 to 23 percentages¹⁶. Important concern in diagnosis of pyospermia is that immature germ cells in simple staining are resembled to leukocytes, in the form that, they called "Round cell" in overall. 1:3 of patients with "Round cell" in their semen, were involved in pyospermia, and in others, just germ cell was seen. The most suitable differential test is peroxide test that is used in laboratory for report of results^{17,18}.

Although both of infection and infertility suggested to be attributed to pyospermia, one should attend that many of pyospermia patients had no infection in their genital tract. Other case-control studies showed that there were not considerable differences between fertile and infertile couples exist. This can be attributed with direct or indirect existence of WBC in semen samples. Laboratory results of case group showed that 60% of them had other various seminal disorders except pyospermia¹⁹.

Impact of sexual behavior on prevalence of genital

Mycoplasma: Prevalence pattern of genital infections in a community is greatly contributed to sexual behavior²⁰ and genital *Mycoplasma* are not exceptions in this regard. Multiple partners, especially with someone who are multi partner themselves, resulted in grant spread of genital infections that is called “ping pong” phenomenon. Because of differences between community in regard of believes, demography and population that are contributed to sexual behaviors, we cannot consider the prevalence of genital infection of other country as the same as ours, in the other hand, both case and control group, using in this study, are not good statistic population to reflect the sexual behavior of our community.

Control group has the fertile men who referring to health center in order to vasectomy, and all of them are married that decided to this, due to responsibility to their families' condition. From the referral cases of Royan institution, number of cases with more than one partner was rare and no cases with sexual behavior beyond ethical frame exist.

Relationship between immunity and pathogenesis

of **Mycoplasma:** One of the special characteristic of *Mycoplasma* is the interaction of one's immunity and its pathogenesis^{21,22}. *Mycoplasma pneumonia* was first extracted from HIV positive patients²³⁻²⁵. *Mycoplasma pneumonia* is important etiology of pneumonia in 5-15 ages and with the maturation of immunity system, its pathogenesis reduced. So, pathogenesis of *Mycoplasma* and its other pathogen species is contributed to the immunity quality. Immunity quality, itself, is contributed genetic and innate characteristics, environmental pollutions, underlying disease and even emotional stress^{21,22}. Therefore, in interpretation of *Mycoplasma* prevalence in infertile patients, the quality of immunity should be considered. One solution to remove this configuring factor, is matching of case and control groups in regard of immunity level in the future study which should be supported with some immunologic assay for determining immunity quality and measuring tests.

Conclusion

From all of above, we can conclude that probability

of genital infection, at least, in these studies group, is very lower than other communities' reports. (If cannot absolutely considered for all community of Iran).

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