

# A Study on Infertility of Males Infected with *Mycoplasma hominis* with Reference to Sperm Morphology

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## Abstract

**Objectives:** The main objective of this study was to investigate the effect of *Mycoplasma hominis* infection on the morphology of sperms and its association with the infertility of men. The patients were referred to the Urology Departments of Mosul General Hospital and Soran Hospital in Mosul and Erbil respectively. **Methods:** The present study was carried out from April 2019 to March 2020 and the number of the patients group was 108. The patients aged 20 to 60 years. Semen was collected from infertile men of a couple that female failed to become pregnant after one year of regular and unprotected intercourse of marriage and submitted for seminal fluid analysis as well as for bacteriological investigations. **Results:** *M. hominis* was detected in 14 semen specimens (12.9%) from the infertile men. The teratozoospermia, normozoospermia, asthenoteratozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, oligozoospermia, oligoasthenozoospermia and leukospermia were seen among patients examined. Statistically, there were no significant differences between these forms of infected infertile men and non-infected infertile men ( $P > 0.05$ ). **Conclusions:** The results of present study demonstrated that the genital *Mycoplasma hominis* seems to be widespread among male partners of infertile couples in Iraq. The present data did not show any significant differences between forms of the sperm concentration and sperm morphology related to the infection by *M. hominis*.

## Keywords

Male, Infertility, Sperms, *Mycoplasma hominis* Infection, Iraq

## 1. Introduction

A history of sexually transmitted diseases (STDs), urinary tract infections (UTIs)

or other genitourinary inflammatory processes may impair the production and quality of sperm and may cause obstruction of the reproductive tract [1]. STDs, such as *Chlamydia* and *Mycoplasma* are associated with decreased sperm counts and higher sperm DNA fragmentation that recovers with antibiotics [2] [3] [4]. Microbial agents impair fertility by causing an obstruction of the male reproductive system, a testicular damage affecting the whole spermatogenesis, sperm cell function impairment, and/or agglutination of motile spermatozoa [5] [6]. *Ureaplasma urealyticum* and *Mycoplasma hominis* are the most common pathogens. The prevalence of *U. urealyticum* has been reported to range from 5% to 42% of the semen samples of infertile men [7]. Several mycoplasmas have been isolated from the urogenital tract and are natural inhabitants of male urethra contaminating the semen during ejaculation. They can cause infections by sexual contacts and for this reason they are often referred as “sexual mycoplasmas” [8] [9].

Chronic prostatitis or epididymorchitis due to either atypical or typical bacterial infections can lead to leukospermia. The presence of these white blood cells in the semen can result in increased sperm DNA fragmentation thought to be the result of increased reactive oxygen species [3] [10] [11]. Microbial agents impair fertility by causing an obstruction of the male reproductive system, a testicular damage affecting the whole spermatogenesis, sperm cell function impairment, and/or agglutination of motile spermatozoa [3] [7]. The prevalence of *U. urealyticum* has been reported to range from 5% to 42% of the semen samples of infertile men [12]. Several mycoplasmas have been isolated from the urogenital tract and are natural inhabitants of male urethra contaminating the semen during ejaculation. They can cause infections by sexual contacts and for this reason they are often referred as “sexual mycoplasmas” [13] [14] [15]. The present attempt is to assess the possible association between male infertility and infection with *Mycoplasma hominis*.

## 2. Materials and Methods

### 2.1. Patients

The present study was carried out from April 2019 to March 2020 and the number of the patients group was 108. The patients aged between 20 to 60 years (average was 32 years). Semen was collected from infertile men of a couple that female failed to become pregnant after one year of regular and unprotected intercourse of marriage and submitted for seminal fluid analysis as well as for bacteriological investigations. Men with genital anomalies (such as varicocele), who had received cefotaxime (2 g/day) during the previous third day of the study were excluded.

### 2.2. Samples

All samples were obtained in the laboratory through masturbation in clean containers, after 5 days of abstinence, with previous washing of the hands and penis with bactericidal soap. The liquefaction of semen was made after 30 min of col-

lection at the incubation of 37°C.

### 2.3. Semen Analysis

Semen analysis was done with indicators like sperm concentration, morphology and motility in all the patients and parameters like total sperm count, motility and presence or absence of abnormal forms were seen. Before collection, the patients were advised for three days of abstinence. Semen samples were collected in the laboratory room in a clean, dry, biologically inert container. In case of oligospermic or azospermic patients, three semen samples were collected on alternate day and thorough examination was carried out. Spermatozoa were counted using the haemocytometer chamber under high power in all four white blood corpuscles (WBC) square using semen diluting fluid consisting of sodium bicarbonate and formalin in distilled water with 1:20 dilution [10] [14] [16] [17] [18]. The sperm counting was carried out same as WBC counting. As the name implies a semen specimen containing no spermatozoa on at least two examinations of different times was considered to be azospermic. Before stamping azospermic, the sample must be centrifuged and check the pellet for sperms as well [19].

### 2.4. Isolation of *Mycoplasma hominis*

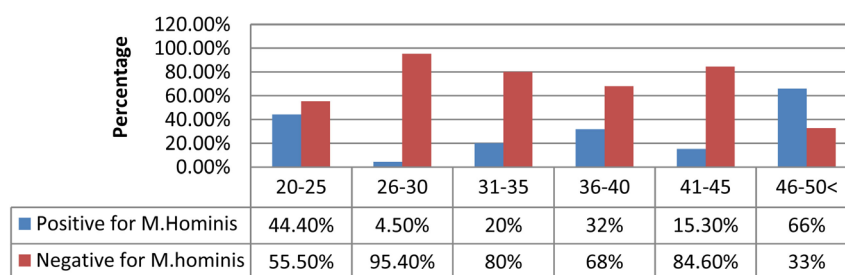
0.1-mL quantity was taken from Ureaplasma transport medium (UTM) containing semen specimen. This sample was inoculated into arginine and phenol containing broth and incubated in sealed tightly tubes at 35°C for 5 days in a moist atmosphere with 5% CO<sub>2</sub>. If the arginine broth changed from red to violet color, the broth was subcultured onto *Mycoplasma hominis* agar plates. The inoculated plates were incubated in a very moist atmosphere with 5% CO<sub>2</sub> at 35°C for 5 days. The suspected growth of mycoplasmal colonies was observed in the agar surface under 40× magnification which appeared as egg fried-like shaped embedded in the agar [20].

### 2.5. Statistical Analysis

Tables, pie charts and bar charts were used to display the findings of this analysis. Normal distribution variables were expressed as mean ± standard deviation utilizing the SPSS version 14.5 computer software Statistical Package for Social Sciences for statistical analyses concerned. Chi-square scale was also used with *P*-value greater than 0.05.

## 3. Results

One hundred-eight infertile men were included in the present study. The patients were subdivided according to their age into six groups. The ages ranged between 20 to 60 years with a peak at the age of 20 to 25 years. Positive sample for *Mycoplasma hominis* of the semen was most commonly seen among the age groups of 36 to 40 years old. No significant difference was seen between age groups and *M. hominis* infection and the *P* value was 0.27 (Figure 1).



**Figure 1.** Relationship between the age groups and *mycoplasma hominis* infection.

The technique used for culture of *Mycoplasma hominis* to be isolated from the semen specimens of 108 infertile males showed that 12.9% were infected with the present bacteria only (**Figure 2**).

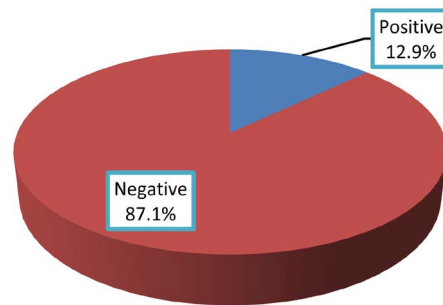
The present study revealed that different types or forms of semen among the infertile males studied were seen. These forms were classified as teratozoospermia. Normozoospermia, asthenoteratozoospermia, oligoasthenoteratozoospermia, asthenozoospermia, oligozoospermia, aspermia and oligoasthenozoospermia and their frequencies were 28.7%, 25%, 19.4%, 9.2%, 7.4%, 3.7%, 3.7% and 2.7% respectively as shown in **Figure 3**.

In the current study the association between frequency of *M.hominis* infection and semen types was shown in **Table 1**. The highest incidence of mycoplasma infection was seen among patients with teratozoospermia and number infected was 4 (3.7%) but oligoasthenozoospermia, aspermia and oligospermia did not show infection with the bacteria present. Statistically, there was a non-significant difference between semen type and *Mycoplasma hominis* infection among the infertile men ( $P = 0.86$ ) using Chi-square test as shown in **Table 1**.

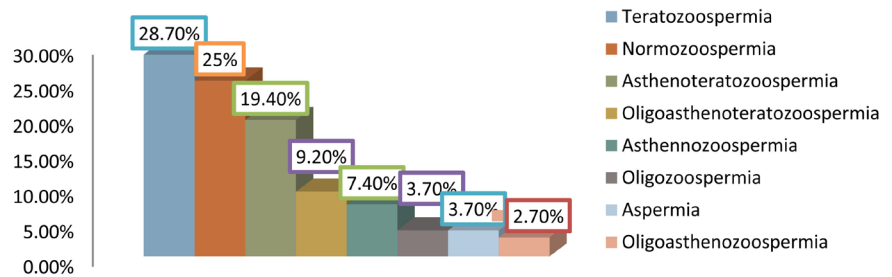
The leukospermia was positive among 13.8% of study cases and infected infertile men was 1.8% of cases studied. Statistically, there was a nonsignificant difference between leukospermia and infected infertile men ( $P = 0.73$ ) using Chi-square test as shown in **Table 2**. Leukospermia was clearly demonstrated in **Figure 4**.

The present study revealed that more than 39 % of the sperms concluded were abnormal in their morphology and the normal sperm frequency was 56.6% (SD  $\pm 0.495$ ) as shown in **Figure 5**. Both vacuolated and small acrosomes of the abnormal sperm head showed a frequency of 14.8%. Different forms of sperms like tapered, rounded, vacuolated and small acrosome were also found among patients with mycoplasma infections and their numbers were 3/15, 3/15, 1/15 and 3/15 respectively (**Table 3**). Statistically, the abnormal sperm head did not significantly differ with respect to *M. hominis* infection ( $P = 0.47$ ). Round and small or tapered heads of sperms were demonstrated in **Figure 6** and **Figure 7** respectively.

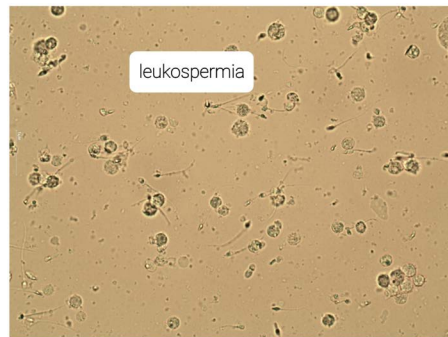
The present study showed that normal necks of sperms were represented by almost 50% of the total cases studied. Other abnormal necks which were with thick insertion, asymmetrical, bent and thin insertion were seen and their frequencies were 25%, 9.3%, 8.3% and 4.6% respectively. Aspermia was also



**Figure 2.** Positive culture for *Mycoplasma hominis*.



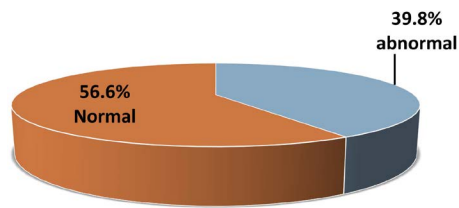
**Figure 3.** Semen types.



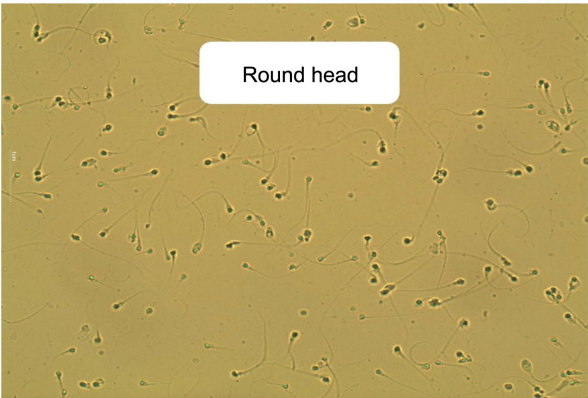
**Figure 4.** Shows the association of *Mycoplasma hominis* infection and sperms with reference to leukospermia ( $\times 400$ ).

**Table 1.** Correlation between semen types and *Mycoplasma hominis* infection.

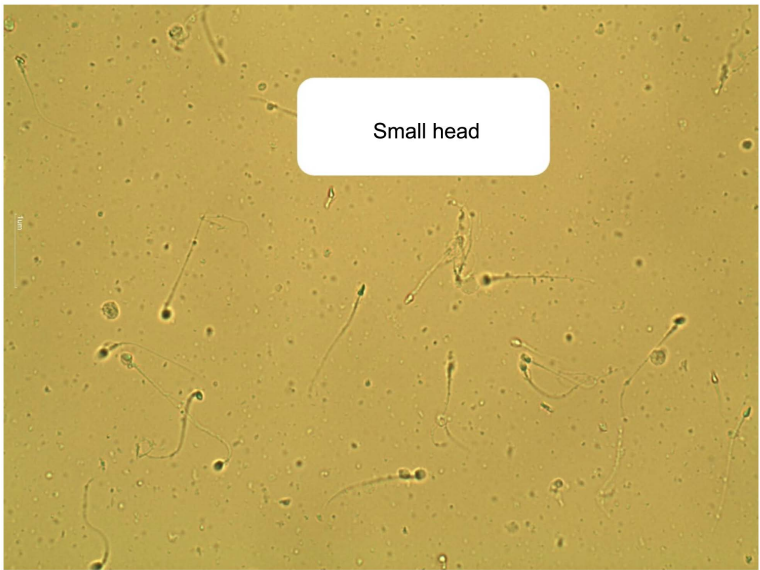
Semen types	<i>Mycoplasma hominis</i>			P value
	Positive (%)	Negative (%)	Total (%)	
Oligoasthenozoospermia	0 (0%)	3 (2.7%)	3 (2.7%)	<b>0.86</b>
Aspermia	0 (0%)	4 (3.7%)	4 (3.7%)	
Oligozoospermia	0 (0%)	4 (3.7%)	4 (3.7%)	
Asthenozoospermia	2 (1.8%)	6 (5.5%)	8 (7.4%)	
Oligoasthenoteratozoospermia	2 (1.8%)	8 (7.4%)	10 (9.2%)	
Asthenoteratozoospermia	3 (2.7%)	18 (16.6%)	21 (19.4%)	
Teratozoospermia	4 (3.7%)	27 (25%)	31 (28.7%)	
Normozoospermia	3 (2.7%)	24 (22%)	27 (25%)	
<b>Total</b>	<b>14 (12.9%)</b>	<b>94 (87.1%)</b>	<b>108 (100%)</b>	



**Figure 5.** Morphology of sperm.



**Figure 6.** Round head sperm of infertile male with *Mycoplasma hominis* infection.



**Figure 7.** Sperm with small head of the infertile male with *Mycoplasma hominis* infection.

**Table 2.** Correlation between *Mycoplasma hominis* and leukaemia.

<i>MH</i>	<i>Leuk.</i>			
		<i>Positive</i>	<i>Negative</i>	<i>Total</i>
<i>Positive</i>		2 (1.8%)	12 (11.1%)	14 (12.9%)
<i>Negative</i>		13 (12%)	81 (75%)	94 (87.1%)
<i>Total</i>		15 (13.8%)	93 (86.1%)	108 (100%)

observed with a frequency more than 3% (**Figure 8**). Statistically, there was a non-significant difference between neck morphology and *M.hominis* Infection ( $P = 0.5$ ) as shown in **Table 4**.

The present study revealed different abnormal tails of sperms among patients with *M.hominis* infection were observed and these were short tail, bent tail, coiled tail and doubled or bifurcated tails and their frequencies of presence were 19.4%, 6.4%, 6.4% and 1.8% respectively (**Figure 9**). Statistically, there was a non-significant difference between tail forms of sperm and *Mycoplasma hominis* infection ( $P = 0.32$ ), shown in **Table 5**. Coiled tail of sperms seen among infertile males with mycoplasmal infection is represented by **Figure 10** and **Figure 11**.

**Table 3.** Relationship between abnormal morphology of sperm head and *Mycoplasma hominis*.

<i>Head</i> \ <i>MH</i>	<i>Positive</i>	<i>Negative</i>	<i>Total</i>
<i>Tapered</i>	3	11	14
<i>Pyriform</i>	0	8	8
<i>Round</i>	3	7	10
<i>Amorphus</i>	0	7	7
<i>Vacuolated</i>	1	15	16
<i>Double head</i>	0	1	1
<i>Small acrosom</i>	3	13	16
<i>Aspermia</i>	0	4	4
<i>Normal</i>	4	28	32
<i>Total</i>	14	94	108
<i>P value</i>		±0.47	

*MH*—*Mycoplasma Hominis*.

**Table 4.** Relationship between abnormal morphology of sperm neck and *Mycoplasma hominis*.

<i>Neck</i> \ <i>MH</i>	<i>Positive</i>	<i>Negative</i>	<i>Total</i>
<i>Bent</i>	1	8	9
<i>Asymmetrical</i>	3	7	10
<i>Thick insertion</i>	7	20	27
<i>Thin</i>	2	3	5
<i>Aspermia</i>	0	4	4
<i>Normal</i>	17	36	53
<i>Total</i>	30	78	108
<i>P value</i>		0.5	

*MH*—*Mycoplasma Honimis*.



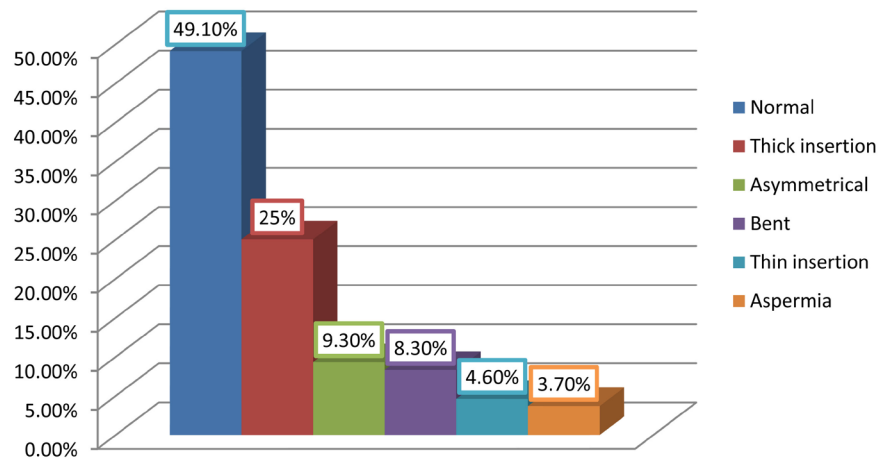


Figure 8. Morphology of sperm neck.

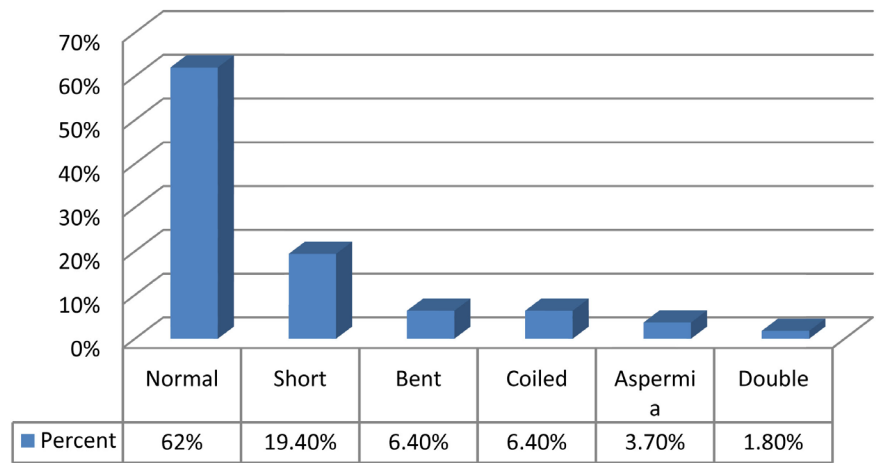
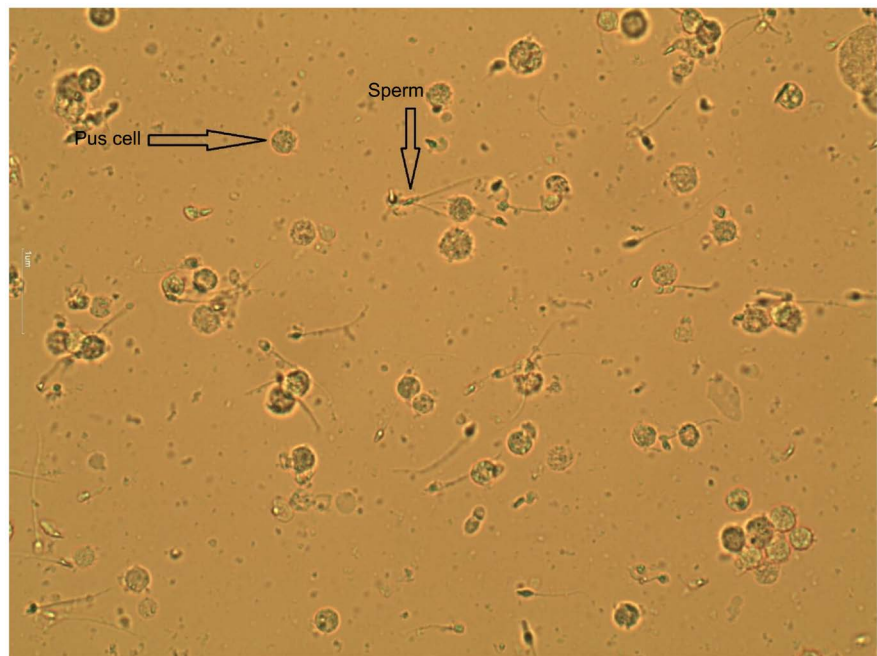


Figure 9. Abnormal tail of sperm.



Figure 10. This picture represents coiled tail of sperm infected with Mycoplasma hominis.





**Figure 11.** This picture represents coiled tail of sperm infected with *Mycoplasma hominis*.

**Table 5.** Relationship between abnormal morphology of sperm tail and *Mycoplasma hominis* infection.

<i>Tail</i> \ <i>MH</i>	<i>Positive</i>	<i>Negative</i>	<i>Total</i>
<i>Bent</i>	0	7	7
<i>Coiled</i>	0	7	7
<i>Short</i>	2	19	21
<i>Double</i>	0	2	2
<i>Aspermia</i>	0	4	4
<i>Normal</i>	12	55	67
<i>Total</i>	14	78	108
<i>P value</i>		<b>0.46</b>	

#### 4. Discussion

The present study revealed that almost 13% of the infertile males were infected with the bacterium *Mycoplasma hominis*. On the other hand, other workers like Zinzendorf found *M. hominis* in 23.8% of infertile men in Africa [21]. Whereas, taken determined *M. hominis* in 3% of infertile men in Turkey [22]. Furthermore, Jensen isolated *M. genitalium* from 17% of male patients with urogenital tract infection in Denmark [23]. For comparison, lee recognized *M. hominis* among 14% and 6.3% of infertile and fertile men respectively. The prevalence rates of *Mycoplasma* and *Ureaplasma* are not well established and vary from one study to another. The heterogeneity of prevalence of mycoplasmal urinary tract

infection in different reports can be probably caused by differences in the geographic areas, the sensitivity of the identification method, the condition of the group (fertile/infertile), other infection accompanied agents, the sample size, and the operator expertise [24]. The prevalence of urogenital infection with *Mycoplasma* in studies by Luki *et al.* in Canada, using cultivation method, was more than 50%, which was higher than our results. The reason could be related to prevailing cultural and social factors and differences among countries. Freedom of sexual relationships and the number of sexual partners in these countries can be considered as reasons for the increased prevalence of *Mycoplasma hominis* [25].

Bacteria and/or their fragments might bind to the sperm and stimulate immune system causing damaging of sperms in all anatomic areas of urogenital tract and reproductive system. Most of infertile males have a history of sexually transmitted diseases (STDs) such as gonococcal or non-gonococcal urethritis. Moreover, the asymptomatic infected males will finally infect their partners and cause secondary couple infertility problems. Most of infertile males' wives have asymptomatic STDs, which might be related to their infected husbands [25]. Bacterial infection is one of the major causes of male and female infertility. Several bacterial genera are involved in infertility with the most common and most important of which are *Mycoplasma* species such as *M. hominis* that play an important role in developing infertility by creating infections without clinical symptoms that lead to not referring to the physician and the disease progression [26]. The present study revealed that the maximum incidence of bacterial isolates was reported among the age group of 46 - 50 and 20 - 25 years old. In comparison, the result reported in our locality was almost similar to that concluded elsewhere [27] and in contrast with those reported by Lee *et al.* [28]. These variations might be related to genetic, geographical or immunological differences between patients [27] [29].

The role of genital tract microorganisms as an important etiological factor in male infertility is still a controversial matter. In the present study, the comparison between infected infertile men and non-infected infertile men showed that the prevalence of reduction in motility and sperm concentration showed non-significant difference. The present result was almost similar to those of Al-Sweih *et al.* [16] and Radhouane *et al.* [30]. Moreover, the infection by *M. hominis* caused variations among the morphology of sperms compared to noninfected males but these variations were statistically not significant [31]. The present study revealed a higher prevalence of patients with teratozoospermia in patients with mycoplasmal infection compared to non-infected infertile men. However, the same conclusion was almost seen by Znazen *et al.* [4]. Furthermore, some studies suggested that mycoplasmas have only a marginal role, whereas others showed a primary role of these microbes on sperm morphology and function [32] [33] [34] [35]. Some investigators did not show any significant correlation between *M. hominis* infection and semen quality [36] [37]. Statistically, the present study did not show any significant difference between sperm

count, motility and/or morphology [38] [39]. Previous studies have reported that the presence of mycoplasmas in sperm specimens has no real effect on the semen quality, nor on the leukocyte count as reported by Andrade-Rocha [26] [40]. Moreover, Bacterial infection is one of the major causes of male and female infertility. Several bacteria genus are involved in infertility, the most common and most important of which are *Mycoplasma* species such as *M. hominis* that play an important role in developing infertility by creating infections without clinical symptoms that lead to not refer to the physician and the disease progression [41].

In the present study, the comparison between infected and non-infected infertile men showed that the prevalence of leukospermia was 10% and 15.3% respectively, although non-significant difference between leukospermia and mycoplasma infection. These findings indicated that the presence of *Mycoplasma hominis* in semen was not necessarily associated with leukospermia, in spite of being potentially pathogenic species. The present results were almost similar to those reported by Andrade-Rocha [40] [42], and in contrast with data reported elsewhere [16]. Moreover, Taylor-Robinson and Furr [43] reported that mycoplasmas represent the second most common cause of acute NGU, after *Chlamydia trachomatis*, and perhaps mycoplasmas may play an important role in chronic NGU [44]. Other investigators considered that mycoplasmas are genitourinary tract commensals; thus, their presence suggests silent colonization and not a real infection; for this reason, the majority of infected patients are asymptomatic and the infection cannot be suspected [45]. In contrast, other studies suggested that *M. hominis* may affect the genital tract leading to an inflammatory obstructive process. These findings indicated that the presence of *M. hominis* in semen was not necessarily associated with leukospermia. Thus, present results appear to show that the presence of mycoplasmas reflects silent colonization rather than an infection in infertile patients [14] [46].

## 5. Conclusion

The present study revealed that almost 13% of the infertile males were infected with the bacterium *Mycoplasma hominis*. The present findings indicated that the presence of *Mycoplasma hominis* in semen was not necessarily associated with leukospermia, in spite of being potentially pathogenic species. Bacteria and/or their fragments might bind to the sperm and stimulate immune system causing damaging of sperms in all anatomic areas of urogenital tract and reproductive system. The present data did not show any significant differences between forms of the sperm concentration and sperm morphology related to the infection by *M. hominis*.

## Ethical Approval

Ethical clearance for the study was obtained from the Committee of Higher Studies in College of Medicine, University of Tikrit. The researcher did not in any

way expose participants of the study to physical or psychological harm. Participation in the study was strictly voluntary with the informed consent of participants that guaranteed their right to privacy. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

## Limitations

Single region data of displacement area is not generalized. For this reason a survey of the whole area can reflect the whole region should be done.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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