



THE ROLE MYCOPLASMA IN MEN IN INFERTILITY

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ABSTRACT

This study was aimed to determine the role of *Mycoplasma* in men infertility in Basrah province and diagnosis species bacterial other causative infertility of men, 100 seminal fluid specimens was collected from men who admitted to infertility center in Basrah province from (1/10/2015) to (31/5/2016), besides that (50) semen specimens was collected from that fertility men as control group. Samples will culture on Monophasic-Diphasic-Culture-Setup (MDCS) for isolation *Mycoplasma*. Samples will culture on Blood agar, MacConkey agar and Chocolate agar for isolation bacteria other than *Mycoplasma*. *Mycoplasma* isolated from (48) case and diagnosed five species: *Ureaplasma urealyticum* (37.5%); *Mycoplasma hominis* (20.83%); *Mycoplasma genitalium* (14.58%); *Mycoplasma pirum* (14.58%) and *Mycoplasma fermenting* (12.5%), where the last two species are first recorded in Iraq from seminal fluid. Study recorded that patients from (30-39) years high infertility rate and *Mycoplasma* infection percentage. High percentage of single infection was *U. urealyticum* and *Mycoplasma hominis*. By using Vitek 2 system diagnosed (22) bacterial species from seminal fluid some isolated at first in Iraq. *Mycoplasma* infected in some patients who suffering from Varicose (18.75%) and Diabetes (8.33%) and bladder nerve pressure (4.16%). This study found, specimens without bacterial infection (7%).The (27) urogenital pathogens The microorganisms affect a negative effect on sperm dysfunction and semen quality, it was including *Mycoplasma* infection (for five species) and other bacterial very effected quantity and quality in seminal fluid analysis for infertile men .Semen size was very small also sperms count, besides that affected on sperms morphology and activity. Also found Pus cells and RBC.

KEYWORDS: Infertility, genital pathogens, semen analysis.

INTRODUCTION

Infertility means the inability to have a child after at least 12 months of unprotected sexual intercourse without using any contraceptive or condom use and affect the cause psychological and social of both spouses (Deka and Sarma, 2010) the incidence of male infertility means of a problem in the genital tract lead to an imbalance and lack of fertility (Askienazy-Elbhar, 2005). One of the causes of secondary infertility is the presence of some bacterial species including the most serious present in the reproductive tract and including *Mycoplasma*, *Ureaplasma*, *Neisseria gonorrhoeae* & *Chlamydia trachomatis* that cause inflammation of the urethra Non gonococcal urethritis (NGU).(Taylor–Robinson, 1996).The emergence of inflammation in the male genital indicates the presences of (*Ureaplasma* and *Mycoplasma*) in the lower genital tract of men weak fertility without any signs or symptoms of infection which causes infertility. Also, infect the female urogenital system and cause bacterial vaginosis inflammatory and pelvic inflammatory and infertility and Miscarriage and postpartum fever and meningitis and abscess and premature birth (Taylor Robinson, 1999). *Mycoplasma* considered the youngest free-living bacteria have the ability to replicate autonomously it is unique among the prokaryotes, Where it lacks the cell wall and as such apart from other microorganisms have been placed in the soft skin class Mollicutes (Rivera-Tapia and Rodring –Preval, 2006). This feature is largely responsible including non-staining gram stain it is negative to weak gram stain Where they

are found in the genital tract in both sexes and is linked to the presence of family members Mycoplasmataceae which includes gender (*Mycoplasma* and *Ureaplasma*) with Sexually Transmitted diseases (STD) Where transmitted from an infected person to a healthy person and increasing incidence in the case of lack of or immune suppression or by infection with human immunodeficiency virus (HIV) Especially sex *Mycoplasma penetrans*, and shows that the risk of sexual behavior in the transfer of sexually transmitted diseases (Askienazy-Elbhar, 2005).

MATERIALS & METHODS

Participants

The current study included the collection of semen (150) a married men aged (20-59 year) 100 a man is infertility and 50 fertile men is considered control group by collecting samples in sterile plastic (cup at the hospital) according to the instructions of where to abstain from ejaculation for at least (48 hr) before a routine examination of a sample of semen and then placed in the incubator for (20 min) at least by converting it from a glass to the transparent liquid may be more than one hour if the high viscosity included measurements of volume sperm count and movement of sperm and sperm been collecting samples in the laboratories of infertility center at Ibn Ghazwan hospital for women and obstetrics in Basra Iraq.

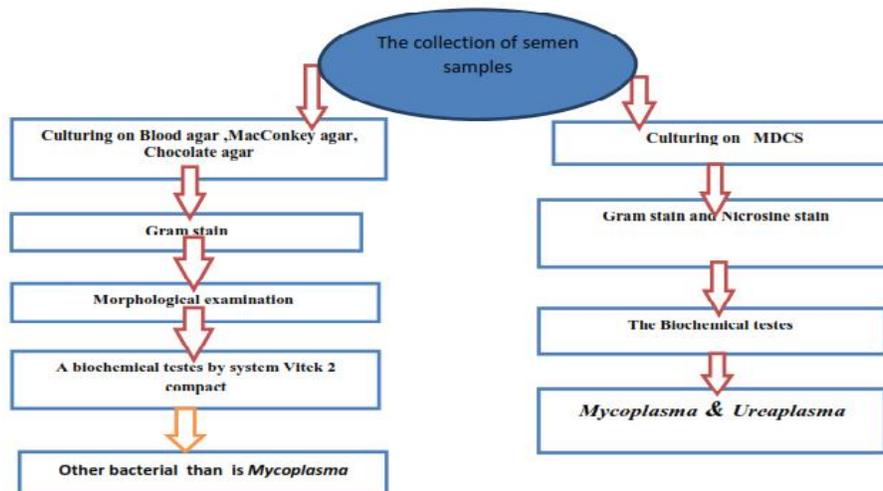
The diagnosis of bacteria *Mycoplasma* & *Ureaplasma* culture samples on the media (MDCS) (Al- Ghazawi, 2001). As well culture all samples on media (Blood, Chocolate, MacConky ager) and it was diagnosed by Vitek

2 system. Rapid identification (three hrs) (Cheng *et al.*, 2010).

Semen collection

All men in the current study were instructed to abstain from masturbation or sexual intercourse for 5 days before attending the clinic up arrival at the clinic the men were asked to collect samples by masturbation on site into standard sterile containers know to be free of cytotoxic

effects on human spermatozoa (WHO,2010). Before semen collection the men were asked to wash their hands and genitalia with soap and running water. Soon after semen collected the samples were promptly liquefied at (37 °C) for about (30 min) in an incubator before analysis. Thereafter, samples were subjected to semen analysis as recommended by WHO (2010).



Flow chart showing isolation and identification of *Mycoplasma* and bacteria other than *Mycoplasma*

RESULTS

The current study included (100) infertile men it is appeared they signs of infection Inflammation Tract Urinary Genital (TUG) and (50) fertile men, the sample taken from the semen of infertile men and men fertile (control group) among infertile men with (48) sample showed a positive result on the presence of bacteria *Mycoplasma* and bacteria *Ureaplasma urealyticum* and the rate (48%) , The rest of the samples that gave a negative result with (MDCS) Which special *Mycoplasma* inhibits growth of other microorganisms, Other bacteria was diagnosed for the first time on of Iraq through the use of system Vitek 2 and recoreded the rate (61%). Is this the study the first the study of the level of Iraq where isolate five types of bacteria *Mycoplasma* of semen in men infection infertility represented bacteria *U. urealyticum*,

M. hominis, *M. genitalium*, *M. pirum* , *M. fermentans* on the media (MDCS). Table number 1 indicates the presence of types *Mycoplasma* in men infertility and men fertility (control). The highest injury for class bacterial Mollicutes as it recorded the highest percentage of the presence of bacteria *Ureaplasma urealyticum* (18 infection) 37.5% of which 14 infection single (77.77%) and (4 infection mixed with other bacteria) two infection with *Staphylococcus hominis* (11.11%) and one infection with *E. coli* (2.08%) and one infection with *Staphylococcus warnerii* (2.08%). Followed by the percentage of presence *Mycoplasma hominis* (10 infection) 20.83%, seven infection single (70%) and (3 infection mixed with other bacteria) one infection with *E. coli* (10%) and one infection with *Granulicatella adiacens* (10%) and one infection with *Staphylococcus warnerii* (10%).

TABLE 1: prevalence of *U. urealyticum*, *M. hominis*, *M. genitalium*, *M. pirum* , *M. fermentans* in infertile men compared with fertile men (control).

	Infection <i>reaplasma</i> <i>urealyticum</i> n. (%)	Infection <i>Mycoplasma</i> <i>hominis</i> n. (%)	Infection <i>Mycoplasma</i> <i>genitalium</i> n. (%)	Infection <i>Mycoplasma</i> <i>pirum</i> n. (%)	Infection <i>Mycoplasma</i> <i>fermentans</i> n. (%)
The study sample (100)	18(37.5%)	10(20.83%)	7(14.58%)	7(14.58%)	6(12.5%)
Control sample (50)	-	-	-	-	-
Total (150)	18	10	7	7	6

It was for the first time to record the type bacteria in semen samples of infertile men represent a kind *Mycoplasma pirum* (7 infection) 14.58%, three infection single (42.82%) and (4 infection mixed with other bacteria) one infection with *Enterobacter cloacae* (14.28%) and one infection with *Aeromonas salmicidae*

(14.28%) and one infection with *Staphylococcus hominis* (14.28%) and one infection with *Staphylococcus haemolyticus* (14.28%) As well as record the injury and the ratio for the presence of *Mycoplasma genitalium*, two infection single (28.57%) and (5 infection mixed with other bacteria) two infection with *Granulicatella elegans*

(28.57%) and one infection with *Staphylococcus haemolyticus* (14.28%) and one infection with *Staphylococcus epidermidis* (14.28%) and one infection with *Staphylococcus warnerii* (14.28%). The other type was diagnosed in the current study bacteria *Mycoplasma fermentans* (6 infection) 12.5%, four infection single (66.66%) and (2 infection mixed with other bacteria) one infection with *Vibrio cholerae* (16.66%) and one infection with *Kocuria kristinae* (16.66%). Also it has been diagnosed with the following bacterial species by Technology system Vitek 2: *Vibrio cholera* (1.63%) and *Aeromonas salmonicida* (1.63%) and *Morganella salmonicida* (1.63%) and *Enterobacter cloacae* (1.63%)

and *Enterococcus faecium* (1.63%) and *Alcaligenes faecalis* (1.63%) and *Staphylococcus xylosus* (1.63%) and *Micrococcus lutes* (1.63%) and *Granulicatella adiacens* (1.63%) and *Kocuria varians* (1.63%) and *Granulicatella elegans* (3.27%) and *Streptococcus pneumoniae* (3.27%) and *Klebsiella pneumoniae* (3.27%) and *Kocuria kristinae* (3.27%) and *Kocuria rosea* (4.91%) and *Pseudomonas aeruginosa* (6.55%) and *Staphylococcus warnerii* (6.55%) and *Escherichia coli* (8.19%) and *Staphylococcus hominis* (8.19%) and *Staphylococcus epidermidis* (8.19%) and *Enterococcus faecalis* (9.83%) and finally bacteria *Staphylococcus haemolyticus* (18.03%).

TABLE 2: *Mycoplasma* patients with a single causative agent or associated with other bacteria is non – *Mycoplasma*

Type of <i>Mycoplasma</i>	Type bacteria of non- <i>Mycoplasma</i>	The number of cause
<i>U. urealyticum</i>		14(77.77%)
<i>U. urealyticum</i>	<i>Staphylococcus hominis</i>	2(11.11%)
<i>U. urealyticum</i>	<i>E. coli</i>	1(5.55%)
<i>U. urealyticum</i>	<i>Staphylococcus warnerii</i>	1(5.55%)
<i>M. hominis</i>		7(70%)
<i>M. hominis</i>	<i>E. coli</i>	1(10%)
<i>M. hominis</i>	<i>Granulicatella adiacens</i>	1(10%)
<i>M. hominis</i>	<i>Staphylococcus warneri</i>	1(10%)
<i>M. genitalium</i>		2(28.57%)
<i>M. genitalium</i>	<i>Granulicatella elegans</i>	2(28.57%)
<i>M. genitalium</i>	<i>Staphylococcus haemolyticus</i>	1(14.28%)
<i>M. genitalium</i>	<i>Staphylococcus warneri</i>	1(14.28%)
<i>M. genitalium</i>	<i>Staphylococcus epidermidis</i>	1(14.28%)
<i>M. pirum</i>		3(42.85%)
<i>M. pirum</i>	<i>Enterobacter cloacae</i>	1(14.28%)
<i>M. pirum</i>	<i>Aeromonas salmonicidae</i>	1(14.28%)
<i>M. pirum</i>	<i>Staphylococcus hominis</i>	1(14.28%)
<i>M. pirum</i>	<i>Staphylococcus haemolyticus</i>	1(14.28%)
<i>M. fermentans</i>		4(66.66%)
<i>M. fermentans</i>	<i>Vibrio cholera</i>	1(16.66%)
<i>M. fermentans</i>	<i>Kocuria kristinae</i>	1(16.66%)
		48

$P < 0.05$, $\chi^2 = 293.217$.

Table number 2 indicates injuries single of *Mycoplasma* and mixed with the rest other bacteria. Either table number 3 indicates types of bacterial isolated of the study the current. And shown the results of analysis statistical the presences of differences between to ratios the percentage of the number of cases infection the single and mixed under the the level of probability $P < 0.05$, $\chi^2 = 293.217$. Table number 3 shows the cases infection for all types bacteria isolate differences under the level of probability $P < 0.05$, $\chi^2 = 320.699$. Ranged the preparation of sperm count fluid intended for men infertility Azoosperm) Ranged preparation volume (0.5-7.5). $100 \times (10^6)$

Also ranged preparation number of forms of sperm normal between (Azoosperm -97) and ranged preparation number of forms of sperm abnormal between (0-97) was types sperm of species A between (0-40) either number sperm of species B between (0-65) and types of species C between (0-70) either number sperm of species D between (0-100), either number of RBC. Preparation (0-4) also found Pus in semen in men infertility preparation between (0-6) .either pH semen was all samples Alkaline, table number 4 shows seminal fluid analysis of patients who infected with *Mycoplasma*.

TABLE 3: Bacteria isolated in the current study

Bacteria isolated type	Number and percentage
<i>Mycoplasma</i> spp.	48(48%)
<i>Staphylococcus haemolyticus</i>	11(11%)
<i>Enterococcus faecalis</i>	6(6%)
<i>Staphylococcus epidermidis</i>	5(5%)
<i>Escherichia coli</i>	5(5%)
<i>Staphylococcus hominis</i>	5(5%)
<i>Pseudomonas aeruginosa</i>	4(4%)
<i>Staphylococcus warneri</i>	4(4%)

<i>Kocuria rosea</i>	3(3%)
<i>Klebsiella pneumoniae</i>	2(2%)
<i>Streptococcus pneumoniae</i>	2(2%)
<i>Kocuria Kristinae</i>	2(2%)
<i>Granulicatella elegans</i>	2(2%)
<i>Enterococcus faecium</i>	1(1%)
<i>Vibrio cholera</i>	1(1%)
<i>Aeromonas salmonicida</i>	1(1%)
<i>Morganella morganii</i>	1(1%)
<i>Enterobacter cloacae</i>	1(1%)
<i>Alcaligenes faecalis</i>	1(1%)
<i>Granulicatella adiacens</i>	1(1%)
<i>Kocuria varians</i>	1(1%)
<i>Staphylococcus xylosus</i>	1(1%)
<i>Micrococcus lutes</i>	1(1%)

$P < 0.05$, $\chi^2 = 320.699$.

TABLE 4: shows the information medical examination presence types of *Mycoplasma*

	<i>U. Urealyticum</i>	<i>M. Hominis</i>	<i>M. genitalium</i>	<i>M. Pirum</i>	<i>M. fermentans</i>
Count	Azoosperm- 100×10 ⁶	55-×10 ⁶ 80×10 ⁶	Azoosperm- 75×10 ⁶	Azoosperm- 100×10 ⁶	40-×10 ⁶ 85×10 ⁶
Volume	1-60	1.5-5.0	1.5-5.0	0.5-3.5	1.5-4.0
Normal	1-60	Azoosperm-97	Azoosperm-60	Azoosperm-65	25-50
Abnormal	0-90	50-97	0-90	0-80	50-75
A	0-15	0-40	0-15	0-10	0-30
B	0-65	0-60	0-45	0-55	0-55
C	0-70	0-25	0-51	0-40	0-35
D	0-100	0-50	0-80	0-60	0-80
RBC.	0-4	0-10	0-2	0-2	0-3
Pus	0-3	0-4	0-4	0-4	0-6
PH	Alkaline	Alkaline	Alkaline	Alkaline	Alkaline

DISCUSSION

Associated with the presence of bacteria (*Mycoplasma*) in samples semen with changing biological in plasma sperm thus will weaken the possibilities of sperm in its ability to fertilize egg (Gonzales, 2001). Male urogenital tract infections are discussed as one of the factor etiological for male's infertility worldwide (Keck *et al.*, 1998). The presence of bacteria semen happens interaction between the fatty acids non- saturated located in membrane sperm (Atig *et al.*, 2012), and related of interferon this cause in the emergence of distortions testes and seminal (Jacobó *et al.*, 2011).and related amounts large of cytokinase working on the perpetuation of inflammation and caused infertility(Qu *et al.*, 2013;Fracsek *et al.*, 2015).Also that the presence of bacteria *Mycoplasma* works to shell movement sperm by Lipo Poly Saccharide (LPS) in the installation of sperm and related factors decomposition *B*-haemolysins – *a*-haemolysins and factors palsy sperm Immobilization Factors (SIF) (Agarwal *et al.*, 2011; Prabha *et al.*, 2011).

Thus will be less than his sperm activity and less activity mitochondrayia mide pices sperm (Kaur and Prabha, 2013). Presence the bacteria in semen Leads to a non-equilibrium between reaction oxygen specific (ROS) and the mechanism of infertility come through adhesion bacteria with samples semen men infertility head a sperm and mide pices and tail (Agarwal *et al.*, 2014).In this study nearly half of patients with infertility almost kano suffering from the presences of bacteria *Mycoplasma* indicating importance the exclusion of these infections and which are not detected by the tests microbial routine only by among culture special by the (Rosemond *et al.*,

2006). Also the types of bacteria of *Mycoplasma* the production of toxins H2S2 and enzymes Phospholipase(Fraczek *et al.*, 2007). The presence of bacteria *Mycoplasma* and *Ureaplasma* in semen works the reducing the elements of micro-like (Zinc and Selenium) these elements be effective in the body's defenses against operations oxidation which generated by the presence of bacteria (Fraczek *et al.*, 2007). As it happens interaction between the components of sperm and components flagella bacteria (Fanibunda *et al.*, 2008).

Recorded bacteria *Ureaplasma urealyticum* over the spread of the rest of bacteria other the proportion of (37.5%) the cause of infertility the resulting due to the presence of bacteria *Ureaplasma* is through the decomposition of urea to ammonia the accumulation of ammonia cause toxicity and adhesion with a sperm reach to mide pice thus will invades cytoplasm head and tail sperm (Reichart *et al.*, 2000) and *M. hominis* (20.83%) and agrees with the result (Salmeri *et al.*, 2012; Cunningham *et al.*, 2013) .At this study other *Mycoplasma* were isolated such as *M. pirum* (14.58%) , and *M. genitalium* (14.58%) like the studies of (Mahony *et al.*, 1997) and result (Hammami *et al.*, 2007; Huang *et al.*, 2015) Also *M. fermentans* (12.5%).

Also ranged preparation number of forms sperm between (Azoosperm -97) and ranged preparation number of forms of sperm abnormal between (0-97) was type's sperm of species A between (0-40) either number sperm of species B between (0-65) and types of species C between (0-70) either number sperm of species D between (0-100), either number of RBC. Preparation (0-4) Also found Pus in semen in men infertility preparation between (0-6) .either pH semen was all samples Alkaline .And in the study

current was observed low sperm count for reducing the natural necessary fertilization egg (Mazzoli *et al.*, 2010). In case nature you must ranging volume (2-6Mm).and volume of the fluid in this study ranged between (0.5-7.5Mm) (Alekw *et al.*, 2013). In cases the ideal you must be form sperm in samples semen of the of a natural more than 70% either in the study current not exceed the 50% (CLSI, 2014). You must be a sperm free of (RBC. and Pus) as the its presences indicates on the presence of inflammation and bleeding because of bacteria and samples semen the present study was a container on (RBC. and Pus) indicating an inflammation and bleeding due to the presence of bacteria (Agrawal *et al.*, 2014). The samples control has not been container on any type bacterial because the presence of bacteria is working on a negative impact on the quality and quality of the number and volume of semen (Lingwood *et al.*, 1990). Was diagnosis bacteria and other non - *Mycoplasma* (22) species through the first time at the level of Iraq by using the technique system device Vitek 2 with card (GN- RST) bacterial negative gram stain and (GP-RST) bacterial positive gram stain technology this device a very high as the diagnosis and a quick and results of the same level of high precision as it was the results of the diagnosis very high ranged between the excellence (88.52%) and very good (11.47%) (NCCLS, 2009). Happens inflammation of urology genital through the transmission bacteria gut and through bacteria located on the urethra as the intervention to the urethra then the bladder then to the kidneys up to the gland prostate cause of inflammation and because the corridor urinary tract is the same passage of sperm so the presence of any type of bacterial affect the quality and quantity of semen and sperm (Schaeffer, 2006).

CONCLOSTION

The(27) urogenital pathogens studied were widespread among infertile and the possibility of them playing a definitive role in the etiology of male infertility in this part of the world appears to be doubtful at this time However because of the association of *Mycoplasma* and *Ureaplasma*, The microorganisms affect a negative effect on sperm dysfunction and semen quality. The presence of bacteria *Mycoplasma* and *Ureaplasma* affect the types of movement sperm and the emergence of shapes of abnormal and affect the total count of sperm and volume of semen in addition to the emergence of RBC and emergence Pus in semen in men in infertility.

RECOMMEDATIONS

A study all the reasons passages leading to me appearance of infertility at the couple while pursuing cases of male infertility in particular , also action bacteriological test for both spouses who suffer infertility before you start taking any antibiotic and provide health laboratory community special to isolate the bacteria that do not isolate isolation routine.

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