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Study of Bacterial infection associated with male infertility in Hillah city-Iraq

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Abstract

Objectives: To identify bacterial species present in the lower genital tract of males and to investigate the relationship with semen quality and male infertility. **Methods**: The microscopic analyses, cultures and ELISA technique of 175 semen and serum specimens, collected over 9 months from males investigated for infertility, were prospectively assessed. **Results**: One hundred and seventy five seminal fluid, blood and serum specimens were collected from men investigated for infertility over a period of 9 months (from April 2011 to December 2011) were analyzed. The seminal fluids and serum of patients mentioned to the laboratory from the fertility clinics of Babylon maternity and children Hospital and outer clinics. The results had shown that from 17 microbial species there are, *Ureaplasma urealyticum* 4.938272 %, *Ureaplasma parvum* 2.160494 %, *Mycoplasma hominis* 2.469136 %, *Mycoplasma genetalium* 5.864198 %, *Chlamydia trachomatis* 9.876543 %, *Streptococcus pyogenes* 8.641975 % , *Staphylococcus aureus* 11.11111 %, *Staphylococcus epidermidis* 12.03704 %, *Staphylococcus saprophyticus* 0.925926 %, *Escherichia* coli 20.06173 %, *Proteus mirabilis* 1.234568 %, *Proteus vulgaris* 2.469136 %, *Klebsiella pneumoniae* 0.925926 %, *Pseudomonas aeuroginosa* 1.54321 %, *Neisseria gonorrhoeae* 2.777778 %, *Toxoplasma gondii* 6.17284 % and *Candida* 6.790123 %. Also the infection with microorganisms revealed that it is higher in azoospermic patients than normospermic group (control).

Keywords: Male infertility, ELISA technique, Bacterial infection.

1. Introduction

There is difference as to the influence of certain microbial infection on male infertility. Several investigators have reported difference types of microorganisms in seminal fluid (Ajabor *et al.* 1999). It was reported that detection of bacteria in semen does not essentially suggest infection because bacterial isolates in seminal fluid may signify colonization of the urethral, contamination, or infection. Enterobacteriaceae, *Chlamydia*, *Ureaplasma* and some gram positive bacteria are the most frequently isolated organisms in industrialized countries (Keck *et al.* 1998). In some parts of the world, oligospermia and azoospermia are most common causes of male infertility which has been reported due to bacterial infections (Ajabor *et al.* 1999 & Megafu 2007).

Urinary tract infections are common in men, and clinicians working with infertility frequently encounter patients with these diseases. Infections include either cystourethritis, caused by trivial urinary bacteria or by sexually transmitted pathogens affecting fertility. The possible relationship between infection and infertility has been the subject of controversy since the second half of the 1970s2, and several therapeutic trials have been initiated since then. The criteria for infection-associated infertility have been laid down in the World Health Organization (WHO) manuals, and several studies of the pathogenesis of reproductive disturbance in infected men have been published in the past decade (Rowe 2000).

An understanding of the link between infection of the ‘accessory sex glands’ and reduced male fertility has been scientifically acquired and diagnostic tools are available, but the results of antibiotic treatment in terms of fertility remain disappointing. The last is probably due to the irreversibility of functional damage caused by chronic infection/inflammation. Therefore, prevention, early diagnosis and correct treatment of infections of the male tract,

both trivial and sexually transmitted, are of pivotal importance (Sergio *et al.* 2007).

According to (WHO), seminal fluid infection was defined as the presence of significant bacteriospermia (⩾103 bacteria/ml ejaculate), detection of *Neisseria gonorrhoeae, C. trachomatis, U. urealyticum*; significant leukocytospermia (106 peroxidase positive leukocyte/ml ejaculate). It therefore follows that if some or all the conditions above are not met, the isolation of bacteria in semen are often regarded as contaminants by most practitioners (Okon *et al.* 2005).

2. Patients and Methods

One hundred and seventy five seminal fluid specimens from men were investigated for infertility over a period of 9 months. These seminal fluids of patients submitted to the laboratory from the fertility clinics of Babylon maternity and children Hospital and outer clinics the practical work was done in the college of science for women in bacteriology, virology and biotechnology labs while serum samples were collected from patients in outer clinics "Ibn Al Nafees laboratory and The Specialist Medical laboratory". Each specimen was collected by patient himself into sterile bottle. The subjects were instructed on how to collect the specimens and submit to the laboratory within one hour of collection. They were told to first pass urine and then wash their hands and penis with soap, then rinse with water prior to masturbation and ejaculation into sterile container. The semen was collected after the patient had abstained from coitus for at least three days. The semen was them cultured on Nutrient, MacConky, Mannitol salt, Thayer martin, Eosin methylene blue, blood, kligler, peptone water, MR-VP, citrate, Sabouraud and chocolate agar media then incubated for 24-48 hours at 37oC, these media used for *Streptococcus pyogenes*, *Staphylococcus* aureus, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Eschirishia coli*, *Proteus mirabilis*, Proteus vulgaris, *Klebseilla pneumoniae*, Pseudomonas aeuroginosa, *Neisseria* gonorrhoeae. Also to confirm the culture results we used Microbial Identification BIOMÉRIEUX VITEK® 2 SYSTEM.

The infective microorganisms were identified by Gram stain and cultivation on media for the cultivable microorganisms, others which are not cultivable microorganisms was detected by ELISA technique such as *Chlamydia*, *Ureaplasma* and *Mycoplasma*. The sperm density, volume, viscosity (liquefaction), the percentage of actively motile sperms, the percentage of abnormal forms, the presence or absence of pus cells were assessed. Analysis was carried out immediately they were received.

3. Results and Discussion Workforce Sizing Plan (WOZIP)

The results are summarized in Tables 1 also in figure 1. Table 1 shows that from 17 microbial species, *Ureaplasma urealyticum* consist 4.938272 %, *Ureaplasma parvum* 2.160494 % , *Mycoplasma hominis* 2.469136 %, *Mycoplasma genetalium* 5.864198 %, *Chlamydia trachomatis* 9.876543 %, *Streptococcus pyogenes* 8.641975 %, *Staphylococcus* aureus 11.11111 %, *Staphylococcus epidermidis* 12.03704 %, *Staphylococcus saprophyticus* 0.925926 %, *Escherichia* coli 20.06173 % , *Proteus mirabilis* 1.234568 % , *Proteus vulgaris* 2.469136 % , *Klebsiella pneumoniae* 0.925926 %, *Pseudomonas aeuroginosa* 1.54321 %, *Neisseria gonorrhoeae* 2.777778 %, *Toxoplasma gondii* 6.17284 % and *Candida* 6.790123 % .

It is estimated that 15% of male infertility is related to genital tract infection (Keck *et al.*1998). From many infectious microorganisms, U. urealyticum is one of the most common species (Wang *et al.* 2006). Since 1967, the ureaplasmas have been shown as an aetiology of male infertility (Radhouane *et al.* 2007), and especially when Friberg and Gnarpe first demonstrated a higher frequency of ureaplasmas in the semen of men with unexplained infertility (76%) compared with fertile men (19%) (Emokpae *et al.* 2009).

In 1999, U. urealyticum biovars 1 and 2 were classified into U. parvum and U. urealyticum, respectively (Kong *et al.* 1999). Most of the previous reported studies have discussed the role of ureaplasmas in male infertility without discriminating between *U. parvum* and *U. urealyticum* (Radhouane *et al.* 2007). In our study, we have used the ELISA assay that can facilitate the identification of *U. urealyticum, U. parvum, M. hominis* and *M. genitalium* in semen specimens. Our results demonstrated that genital mycoplasmas and ureaplasmas seem to be widespread among infertile male patients, as shown respectively by the frequency of 13% and 15.2%. These data are comparable with those reported in previous studies (Andrade-Rocha 2003). *U. urealyticum* was the most prevalent species of *Ureaplasma* genus detected (9.2%) in this study. (Rosemond *et al.* 2006 & Kong *et al.*1999). This wide range might be explained by the diversity of detection methods used for characterizing the studied populations and the technique

used in detection. In our study, *U. parvum* was detected in 3.8% of serum samples. The frequency of this species was lower than that reported by Knox et al. (3.8% vs 19.2%) (Reichart *et al.* 2001). *M. hominis* has been associated with bacterial vaginosis, pelvic inflammatory disease, postpartum fever, and postabortal fever, as well as a number of gynaecological infections (Yoshida *et al.* 2003).

However, its role in non-gonoccocal urethritis (NGU) and in infertility is rarely investigated (Pannekoek *et al.* 2000). The frequency of *M. hominis*, in our study 4.5%, it was comparable to that reported by Andra-Rocha et al. and Radhouane Gdoura et al. but higher than that reported by Rosemond (Deguchi & Maeda 2002). *M. genitalium* was first isolated in urethral cultures from two men with NGU in 1981, although *M. genitalium* has been suggested as a cause of human NGU, the precise role of this mycoplasma in the etiology of NGU remains not established because of the immense difficulty in isolating it from clinical samples (Jensen *et al.* 2004).

Hitherto, *M. genitalium* has seldom been investigated in semen of infertile men. In our study, the frequency of *M. genitalium* 5.864198 % it was higher than that reported by Kjaergaard et al. (Kong *et al.* 1999) (5.864198 % vs 0.9%).

This difference might be explained by the use of different methods for the detection of this bacterium. We have used ELISA that is more sensitive than culture and that can facilitate the detection of *M. genitalium* in clinical samples (Deguchi & Maeda 2000).

In the present study, the frequency of the U. urealyticum was higher than that of *M. hominis*. *U. urealyticum* was also detected more often than U. parvum; these findings were consistent with other studies (Jensen *et al.* 2004).

Previous studies had reported that the presence of mycoplasmas and ureaplasmas in sperm specimens has no real effect on the semen quality, or on the leukocyte count (Emokpae *et al.*2009 & Kong *et al.*1999). Other investigations seem to show that the presence of mycoplasmas reflects a silent infection rather than infection in infertile patients (Reichart *et al.* 2001), even though when the attachment and invasiveness towards human sperm cell has been demonstrated in vitro (Yoshida *et al.* 2003).

In the present study, the comparison of the sperm seminological variables of *U. urealyticum*-positive and *U. urealyticum*-negative infertile men demonstrated no significant differences in sperm seminological variables, which confirms previous findings. Conversely, a relationship between *U. urealyticum* and semen characteristics was observed in some literature (Emokpae *et al.* 2009 & Lackner *et al.* 2006).

The influence or the lack of influence of mycoplasmas and ureaplasmas on seminology may come from the capability of bacterial species to attach to spermatozoa and to affect directly via cellular interactions their vitality, motility, morphology, cellular integrity and their molecular structure or the development of protective immunity to genital infection by the host (population sensitivity to microbial agents) or other host factors (Lackner *et al.* 2006). Semen with *M. hominis* presented a higher mean of leukocytes than semen with negative *M. hominis*. In contrast, the means of leukocyte count of the positive ELISA for *U. urealyticum, U. parvum* and *M. genitalium* were nearly same than the reference value of the WHO manual. These findings indicate that the presence of mycoplasmas and ureaplasmas in semen is not necessary associated with leukocytospermia, and thus, in spite of potentially pathogenic species. Our results are consistent with previous reports (Radhouane *et al.* 2007 & Yoshida *et al.* 2003). Infection with microorganisms revealed that it is higher in azoospermic patients than normospermic group (control) as shown in figure 1 *Ureaplasma urealyticum* (62.5%, 0) U. *parvum* (100 %, 0), *Mycoplasma hominis* (62.5%, 0) *M. genetalium* (63.16%, 0) *Chlamydia trachomatis* (46.8%, 0) respectively. Interestingly, Staphylococcus aureus as causative organism accounted for 20.3% of seminal fluid infection in this study. This ratio is lower than reported by Okon et al. (Radhouane *et al.* 2007 & Yoshida *et al.* 2003) in Maiduguri, where Staphylococcus aureus was isolated from 62.5% of the seminal fluids. Most practitioners dismiss this infection as mere contamination which is assumed to be of no significance. The WHO definition of seminal tract infection does not clearly differentiate between infection, contamination and colonization of the genital tract. Semen that passes through the genital tract is routinely contaminated with Gram positive cocci such as *Staphylococcus*, *Streptococcus* and Diphteroids. It is generally accepted that *Staphylococcus* aureus which is coagulase positive is regarded as pathogenic and should be treated. The presence of this microorganism can no longer be ignored. The longer the infection persist, the greater the damage and loss of germ cells. The rate (percent) of infection increases from normospermic to azoospermic males (Rosemond *et al.* 2006).

According to Bukharin et al. Opportunistic microorganisms cause classical infections of the urogenital tract and subclinical reproductive tract infections. These infections of the seminal fluid lead to decrease in the number of

spermatozoa, the suppression of their motility, changes in their morphology and fertilizing capacity. Our result shows that 58.2% of the males had sperm density below 20 million/ml. The sperm morphology deteriorated progressively in oligospermic to severe oligospermic males. In other words it decreased with decreasing sperm density. Azoospermia was also observed in 58.2% of the study population (Radhouane *et al.* 2007 & Rosemond *et al.* 2006).

The results obtained from questionnaire on infertility assessment showed that most of the semen samples from those infertile male patients belong to 20-36 years old category. The idea that bacterial infection may be partly responsible for male infertility arises from the clinical observation of the patients' male reproductive system (Punab *et al.* 2003 & Bukharin *et al.* 2005).

Male Urogenital Tract Infection is one of the most important causes of male infertility worldwide (Keck *et al.* 1998). Infection processes may lead to deterioration of spermatogenesis, impairment of sperm functions, and obstruction of the seminal tract. In the light of the above, there is the need to institute a microbiological intervention to detect the probable microbial agents. In view of our study, it seems that Leukocyspermia is a poor maker to predict bacteriospermia (Table 2, 3). Consequently in presence or absence of the Leukocytospermia, microbiological investigation should be performed on all semen, as a routine test, from infertile male attending infertility clinics. It should be noted that presence of Urogenital Tract Infection and inflammation posed a danger to the fertility profile of male patient and should be eradicated by antibiotics and anti-inflammatory treatment (Rodin *et al.* 2003).

5. Conclusion

Positive seminal fluid cultures were interpreted with caution, taking into account both raised colony counts of single isolates in the semen. Thus the common misdiagnosis of genital tract infection, based on the presence of seminal bacteria, and unnecessary treatment with antibiotics may be avoided.

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Table 1- Types of microbial infection associated with infertile men and control.

Type of microorganism	No.	%
Ureaplasma urealyticum	16	4.938272
Ureaplasma parvum	7	2.160494
Mycoplasma hominis	8	2.469136
Mycoplasma genetalium	19	5.864198
Chlamydia trachomatis	32	9.876543
Streptococcus pyogenes	28	8.641975
Staphylococcus aureus	36	11.11111
Staphylococcus epidermidis	39	12.03704
Staphylococcus saprophyticus	3	0.925926
Escherichia coli	65	20.06173
Proteus mirabilis	4	1.234568
Proteus vulgaris	8	2.469136
Klebsiella pneumoniae	3	0.925926
Pseudomonas aeuroginosa	5	1.54321
Neisseria gonorrhoeae	9	2.777778
Toxoplasma gondii	20	6.17284
Candida	22	6.790123
Total	324	100

Table 2- Relation between microbial isolates, abnormal sperm morphology and total motility.

Bacterial isolate	Abnormal Sperm morphology (>60%)		Total motility ml < 50 % or more with forward progression or < 25 % or more with rapid progression within 60 minutes of ejaculation	
	No.	%	No.	%
Ureaplasma urealyticum	11	10.37736	6	13.63636
Ureaplasma parvum	4	3.773585	1	2.272727
Mycoplasma hominis	2	1.886792	1	2.272727
Mycoplasma genetalium	5	4.716981	1	2.272727
Chlamydia trachomatis	13	12.26415	4	9.090909
Streptococcus pyogenes	9	8.490566	3	6.818182
Staphylococcus aureus	19	17.92453	5	11.36364
Staphylococcus epidermidis	3	2.830189	0	0
Staphylococcus saprophyticus	7	6.603774	1	2.272727
Escherichia coli	22	20.75472	14	31.81818
Proteus mirabilis	1	0.943396	1	2.272727
Proteus vulgaris	0	0	1	2.272727
Klebsiella pneumoniae	0	0	1	2.272727
Pseudomonas aeuroginosa	1	0.943396	1	2.272727
Neisseria gonorrhoeae	4	3.773585	2	4.545455
Toxoplasma gondii	3	2.830189	1	2.272727
Candida	2	1.886792	1	2.272727
Total	106	100	44	100

Table 3- Age classification, number of isolates, leucocytes and sperm count/mL

Age (years)	No. examined			No. of positive leukocyte			Sperm count (x106) mean		
	Azoo-spermia	Oligo-spermia	Normo-spermia	Azoo-spermia	Oligo-spermia	Normo-spermia	Azoo-spermia	Oligo-spermia	Normo-spermia
21-25	20	20	7	11	7	2	0	1.7	32.3
26-30	19	19	6	13	10	4	0	1.1	30.7
31-35	19	19	6	9	12	2	0	1.4	26.6
36-40	19	19	6	12	7	1	0	1.2	22.5

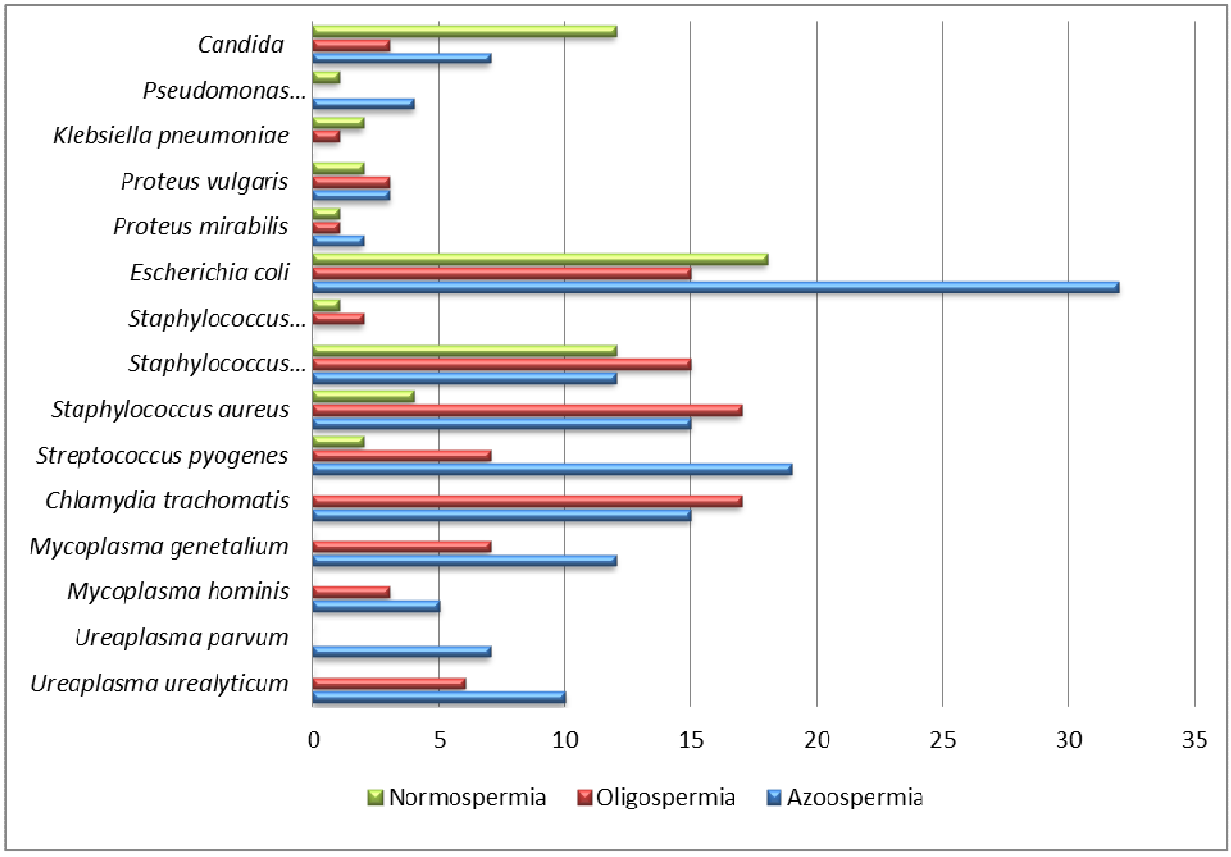

Figure 1: Types of Microbial infection associated with azoospermic, oligospermic and normospermic patients.

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