

GENITOURINARY TRACT INFECTIONS RELATIONSHIP WITH MALE INFERTILITY: A BACTERIOLOGICAL STUDY⁺

دراسة بكتريولوجية للعلاقة بين اخماج المسالك البولية - التناسلية والعقم عند الرجال

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Abstract:

A total of one hundred and twenty eight infertile males (age 20-45 years) with genitourinary tract infections were studied. The pus cells in the semen and urine were determined by the direct microscopic examinations, urine culture and semen analysis with culture. Thirty-seven (27.34%) of the patients have had an azoospermia, eighty patients (62.50 %) have had an oligospermia, and thirteen patients (10.13%) have had a spermatic count in the normal range. The common pathogens were isolated from the semen and the urine samples: *Escherichia coli* (18.35%), *Staphylococcus aureus* (14.49%), Coagulase negative Staphylococci (13.53%) and *Klebsiella spp.* (12.56%) respectively. There is a correlation (Cor=0.89) between the isolates from the semen and the urine. Urinary tract infections were acting as a nidus of infection for seminal tract. Antibiotic susceptibility of pathogenic isolates were shown that the Ciproflaxin, Ceftriaxone, Rifampicin, Gentamycin, and Augmentin (Amoxicillin and Clavulante) as the more effective for the treatment of these infections caused by the Gram positive and Gram negative bacteria as the severe pathogens. The study recommends that the genitourinary tract infection is one of the possible causes for male infertility; also, before the decision about the treatment it is necessary to determine the causative agents of infection.

المستخلص:

شملت الدراسة مائة وثمانية وعشرون من الذكور بعمر ٢٠-٤٥ سنة والذين يعانون من العقم مع أخماج المسالك البولية-التناسلية، بوجود الخلايا القحيبة في السائل المنوي أو الإدرار عندهم. وبأجراء الفحص المجهرى المباشر والزرع البكتيري للسائل المنوي وزرع الإدرار. اظهرت النتائج أن ٢٧,٣٤% من المرضى كان تعداد النطف في السائل المنوي صفراً؛ ٦٢,٥٠% كان عدد النطف أقل من العدد الطبيعي؛ اما الباقين ١٠,١٣% فأن تعداد نطفهم كان طبيعياً. كذلك اظهرت الدراسة ان الممرضات الأكثر شيوعاً المعزولة من الإدرار والسائل المنوي: أيشيريشيا القولون (١٨,٣٥%)؛ المكورات العنقودية الذهبية (١٤,٤٩%)؛ المكورات العنقودية السالبة للتلط (١٣,٥٣%) والكليسيلا (١٢,٥٦%) على التوالي. وجدت علاقة موجبة بين العزلات من السائل المنوي والإدرار، إذ ان أخماج المسالك البولية تعمل كمنبت لأخماج المسالك التناسلية والمنوية عند الرجال. أظهرت الدراسة إن المضادات الحيوية: السبروفلاكساسين؛ السفترياكسون؛ الريفامبيسين؛ الجنتاميسين والايوكمننتين (اموكسيلين و كلافولانت) الأكثر تأثيراً في العزلات المرضية الموجبة والسالبة لصبغة جرام. الدراسة توصي إن عدوى الجهاز البولي

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التناسلي هو احد الأسباب المحتملة لعقم الرجال، أيضا قبل اتخاذ قرار بشأن العلاج من الضروري تحديد العوامل المسببة للعدوى.

Introduction:

The conventional definition of infertility is a one year of unprotected sexual intercourse without establishing a successful pregnancy. Conception normally achieved within this period in 80-90% of couples using no contraceptive measures [1,2], while certain cases of male infertility are due to anatomical abnormalities such as varicoceles, ductal obstructions or ejaculatory disorders, seminal tract infections other than sexually transmitted diseases and tuberculosis [3,4]. Several microorganisms involved in male reproductive failure in different ways, and to dissimilar degrees of statistical association. Infections of the lower genital tract seem to have a little importance, if not, in the unlikely event of an occlusion [5]. However, those involving other parts of the male genitourinary tract, may cause a bacterial colonization of the semen [6]. Krausz, *et al.* [7] demonstrated that about one out of seven European couples suffer from reproductive health disorders in the form of infertility.

Moreover, many external factors are due to male infertility such as environmental toxins, drugs such as Cimetidine (Tagamet), heavy cigarette; alcohol use can be associated with decrease sperm count and reduced fertility [8]. Bacterial infections may often go unobserved but could hamper fertility in several ways. In addition, inflammation and fibrosis could block the passage sperms could be directly damaged and immobilized or the chemical composition of the seminal plasma could be adversely affected. The male genital tract is frequently invaded by bacteria, and it has shown the different kind of impaired sperm functions and constant infertility [9]. The aim of this study is to investigate the role of genitourinary tract infections in the relationship with the male infertility, and antibiotic susceptibility of the isolates to some antibiotic which used for treatment.

Methods:

1: Patients:

One hundred twenty eight patients (128) (age 20-45 years) attending to the infertility clinic at the Al-Hussein General Hospital at Al-Nasseria city for the six months period. Medical and sexual history was taken from each patient; an important fact in the history is genitourinary tract infections with particular attention to previous venereal diseases, post adolescent mumps, epididymo-orchitis, a history of testicular injury, bacterial and viral infections, a surgery in the male genitourinary tracts and taken medication were taken.

2: Sampling:

Seminal fluid samples (average volume 2-5ml) were collected by masturbation in the sterile containers, under aseptic precautions, after three days abstinence. The semen was allowed to liquefy completely and then spermatic count according the World Health Organization (WHO) normal values in the table 1[10].

Table 1: The WHO normal values for the seminal analysis

Volume	2 ml or more
pH	7.2-8.0

Spermatic concentration	20 million /ml or more
Total sperm count	40 million per ejaculate or more
Motility	50% or more
Morphology	30% or more normal forms
Pus cells	Less than 10 per high power field

3: Bacterial cultures:

Semen inoculations were done on two Blood agar, two MacConkey agar, and two Chocolate agar plates, then incubation under the aerobic and the anaerobic conditions at 37 C° for 24 hours. Mid-stream urines were collected for culture, 2-3 days after the semen collection, but never on the same day. The urine samples were cultured in a similar ways of semen culture, then examined for evidence of growth, subculture on solid media and biochemical tests (Indole, Methyl red, Voges-Proskauer, Simmon's citrate, Triple sugar iron, Urease, Oxidase, Motility, Catalase, and Coagulase tests) were done for the identification and confirmation of the bacteria were isolated [11]. Anaerobic bacteria were cultured by plating on selective media containing antibiotics; the plates were incubated under anaerobic conditions in Gas Pack Jar at 37 C° for 48-72 hours.

4: Antibiotic susceptibility:

Antibiotic susceptibility tests were done against the isolates by the standred disc diffusion of ten antibiotics such as: Ampicillin (AMP), Augmentin (AUG), Amikacin (AK), Amoxicillin (AML), Erythromycin (ER), Ceftriaxone (CFR), Rifampcin (RD), Trimethoprim (STX), Gentamycin (GM) and Ciproflaxacin (CP) [12].

5: Statistical analysis:

The results were statistically evaluated using SPSS version 18.0, which is One Way analysis of variance (ANOVA). A *P*-value of 0.05 or less is considered significant ($P \leq 0.05$). The Pearson correlation was also performed using the same program software to estimate the correlation between the groups of the isolates.

The results:

One hundred and twenty eight infertile males were investigated. Epididymal tenderness was presented in the 107 patients (83.59%), including a significant number of pus cells in the semen (more than 10 cells per a high power field), and 21patients (16.41%) did not have bacteria and no pus cells in the semen. Thirty-seven (27.34%) patients have had an azoospermia (spermatic counts were zero), eighty patients (62.50%) have had an oligospermia (spermatic counts were below 40 million /ml) and thirteen patients (10.15%) have had the spermatic counts in the normal range (spermatic counts were above 40 million/ml). One hundred and eleven (11\` of the bacteria were isolated from 107 seminal fluids of infertile patients, were illustrated in table 2, including mix growth (more one bacteria). Gram positive bacteria are including: coagulase negative Staphylococci (14.41%); *S. aureus* (12.61%);

Enterococcus faecalis (8.10%) *Streptococcus agalactiae* (6.30%) and Gram negative bacteria are including: *E. coli* (16.21%); *Klebsiella spp.* (12.61%); *Enterobacter spp.* (10.81%) *Protues spp.* (5.40%), and low frequently of other pathogens as *Candida spp.* (5.40 %); *Neisseria gonorrhoea* (6.30%) were shown respectively. However, no growth was appeared from anaerobic bacteria. The bacteria were isolated from the urine are showing in table 3. The correlation between the isolates finding in the semen and the urine is showing in table 4. Table 5 shows the efficiency of routinely available antibiotics against highly frequencies pathogenic bacteria which confirmed statistically ($p < 0.05$).

Table 2: The frequency and the type of bacteria were isolated from the semen samples.

Bacteria*	No.	%
Gram positive		
Coagulase negative Staphylococci	16	14.41
<i>Staphylococcus aureus</i>	14	12.61
<i>Enterococcus faecalis</i>	9	8.10
<i>Streptococcus agalactiae</i>	7	6.30
<i>Candida sp.</i>	6	5.40
Gram negative		
<i>Escherichia coli</i>	18	16.21
<i>Klebsiella sp.</i>	14	12.61
<i>Enterobacter sp.</i>	12	10.81
<i>Proteus sp.</i>	6	5.40
<i>Neisseria gonorrhoeae</i>	7	6.30
<i>Serratia sp.</i>	2	1.80
Total	111	100

*N=128; positive culture=107; negative culture=21; mix growth=4

Table 3: The total of microorganisms were isolated from urine

Bacteria*	No.	%
Gram positive		
<i>S. aureus</i>	16	16.66
Coagulase negative Staphylococci	12	12.50
<i>Streptococcus agalactiae</i>	8	8.33
<i>Enterococcus faecalis</i>	6	6.25
<i>Candida sp.</i>	3	3.12
Gram negative		
<i>E. coli</i>	20	20.83
<i>Klebsiella spp.</i>	12	12.50
<i>Enterobacter spp.</i>	8	8.33
<i>Proteus spp.</i>	6	6.25
<i>Serratia spp.</i>	3	3.12

<i>Pseudomonas spp.</i>	2	2.08
Total	96	100

* N=128; positive culture=96; negative culture =32

Table 4: The total of the microorganisms were isolated from the urine and the seminal fluid.

Bacteria	Semen culture		Urine culture		Total*	
	No.	%	No.	%	No.	%
<i>E. coli</i>	18	16.21	20	20.83	38	18.35
<i>S. aureus</i>	14	12.16	16	16.66	30	14.49
Coagulase negative Staph.	16	14.41	12	12.50	28	13.53
<i>Klebsiella spp.</i>	14	12.16	12	12.50	26	12.56
<i>Enterobacter spp.</i>	12	10.81	8	8.33	20	9.66
<i>Sterptococcus agalactiae</i>	7	6.30	8	8.33	15	7.25
<i>Enterococcus faecalis</i>	9	8.10	6	6.25	15	7.25
<i>Proteus spp.</i>	6	5.40	6	6.25	12	5.79
<i>Candida spp.</i>	6	5.40	3	3.12	9	4.34
<i>Neisseria gonorrhoeae</i>	7	6.30	0	0	7	3.38
<i>Serratia spp.</i>	2	1.80	3	3.12	5	2.41
<i>Pseudomonas spp.</i>	0	0	2	2.08	2	0.96
Total	111	100	96	100	207	100

*Correlation Coefficient= 0.89, p=0.546

Table 5: The susceptibility pattern (percentage) of the common isolates against the antibiotics.

Bacteria*	AMP	A K	AUG	AM L	ER	CFR	RD	STX	G M	CP
<i>S. aureus</i>	40	36	92	40	35	92	54	35	68	65
<i>E. coli</i>	60	72	65	35	30	70	68	32	72	95
Coaglase negative Staph.	72	56	85	55	50	74	60	54	80	74
<i>Klebsiella spp.</i>	65	70	76	45	45	82	54	40	72	92
<i>S.agalactiae</i>	80	82	82	70	65	88	62	52	58	75
<i>Enterobacter spp.</i>	55	75	75	55	40	85	58	38	77	86
<i>Proteus spp.</i>	56	70	70	45	35	78	52	42	75	82

*P<0.05

Discussion:

It is generally accepted that the diagnoses of infertile couple should be initiated after 12 months of regular unprotected intercourses. However it must be taken into consideration that about half of the couples which do not imagine during the first year will do so during the second year. The diagnoses of the infertile male should include careful medical and reproductive history, physical examination and semen analysis [7]. Despite major advances in the diagnostic work of infertile males, the aetiology of testicular failure remains undefined in about 50% of cases and is referred to as idiopathic infertility [13].

From the results, 16.41% of the infertile patients have given a no growth on the semen cultures, while, remaining 83.59% have one or more types of the bacteria were isolated. The mix growth was found in 3.12% of the all specimens. A total of isolates 111 from 107 patients were undergoing infertility (table 2), this is in agreement with Turek [2] and Keck *et al.* [14] who referred to the seminal fluid that passes through the urethra is routinely contaminated with bacteria, given that 83% of all infertile men will have positive semen cultures. Also, the infections of male genital tract are the important factors for the male infertilities.

The most common pathogens were isolated from the seminal fluids of Gram negative and Gram positive bacteria were *E. coli* (16.21%) and Coagulase negative Staphylococci (14.41%), this is in agreement with others studies [15,16]. Whereas, others researchers have found *E. coli* and Enterococci are the common pathogens in the semen of infertile males [17, 18, 19]. On the other hand, Keck *et al.* [14] demonstrated that Enterobacteria can be found in up to 90% of semen samples depending on the sensitivity of detection methods used.

The urine cultures (table 3) showed growths in 75.00% of the infertile males were tested. While in the 25.00% of the urine samples were no growth, the common pathogens were *E. coli* (20.83%); *S. aureus* (16.66%) and Coagulase negative Staphylococci (12.50%). This is in agreement with the study carried by Mogra *et al.* [15] who had found *E. coli* and *S. aureus* the most common pathogens were isolated from the urine samples in the patients suffering from genitourinary tract infections.

The correlation between the frequency of bacterial isolates findings in the semen and the urine were explained in the table 4. An effort has also been made to correlate spermatic count with the type or number of bacteria was isolated from seminal fluids. The correlation between the bacterial cultures and infertility is at inconclusive [2]. Out of 128 cases of the infertile males, 35 had azoospermia, while 80 revealed oligospermia and 13 cases had spermatic count in the normal range, no definite relationship could be observed between the type of bacteria were isolated from the semen and the spermatic count, but there is a positive correlation found between the pathogens were isolated from the urine and seminal fluid samples (Cor=0.89). This is in agreement with study carried by Mogra *et al.*[15] who had found the urinary tract infections in males were acting as nidus of infection for the seminal tracts. Other, demonstrated to this infection may cause occlusion in the canalicular system of male genital tracts, may damage the epithelial cells involved in the spermatogenesis [20]. Also, it has been ability to destroy the sperm membrane which has an important role in the transportation of the infected agent in to upper genital system lead to infertility [21]. Infection of the male genitourinary tract may contribute to infertility by adversely affecting sperm function, damaging sperm, hampering their motility, altering the chemical composition of the seminal fluid, and causing anatomical obstruction [22, 23]. On the other hand, seminal infection could also be the cause of the chronic urinary tract infection by acting as the reservoir of infection [24]. The bacteria are identified to give urethritis, which is not often

complicated by infections of other parts of the genital tract, including the testes, and in this way can harm male fertility [1].

In the same sense, it appears from results (table 5), that Ciprofloxacin, Ceftriaxone, Rifampicin, Gentamycin, and Augmentin (amoxicillin and Clavulante), there are the first choice of treatment these infections caused by serious pathogens Gram positive and Gram negative bacteria, and other antibiotics with moderate effecting on these pathogens such as Amikacin and Ampicillin (second choice), there is significant differences between them ($P < 0.05$), this is an agreement with other studies [22, 25].

Conclusions:

Infertility is a medical problem of couple. One of the possible causes for male infertility is genitourinary infections. Before the decision about the treatment it is necessary to determine the causative agents of infection which is commonly isolated. However, despite of extended diagnosis efforts for detection of most infectious diseases, the causal link between infection and male infertility has not been established

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