



The Effect of Bacterial Infection on Male Infertility

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Abstract: There are several reasons for altered semen quality and bacteriospermia could be one among them. However, there is no complete agreement on detrimental role of the premises suggesting the bacterial semen infection is associated with male infertility. The aim of this work is to study the semen culture and detection of bacteria in semen as well as their association with semen quality among infertile male. Semen samples were collected from men attending high institute for infertility diagnosis assisted reproductive technology in Baghdad Al-Kadhmiya for the period from June-July 2019. Samples were subjected to culture using standard bacteriological techniques according to WHO guidance. A total of fifty samples were collected. 45(90%) showed bacteriospermia. *Enterococcus* 32% was the most common organism followed by *Klebsiella* spp. 24%, while *proteus* spp. and *staphylococcus* spp. 18% respectively, and other less frequently organism is *E.coli* 16. Standard semen analysis was performed, including volume liquefaction, pH, concentration, morphology, agglutination, round cell count and percentage motility. Results showed the presence of bacteria growth does not impact on their parameters concluding that no definite relationship was established between semen parameters and bacteriospermia.

Keywords: Infertility, Male, Bacteria.

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Introduction

Infertility is a health concern affecting about 10% worldwide population. This term describe inability of couples to achieve conception post one year of regular unprotected sexual intercourse. Which is normally happen within 12 months in 80-85% of couples without determinants (1). Male factors infertility responsible about 50% of all cases (2,3). Male infertility factors include complete absence of sperm, low numbers of sperm, progressive movement and abnormalities in the sperms ability to fertilize an egg (4). There are different causes of male infertility, including spermatogenesis disorders, chronic diseases, Sexually transmitted diseases (STDs) and Male urogenital tract infection (5). The last cause most important condition since

genital tract infection and inflammation have been associated with 8-35% of male infertility cases (6,7).

Bacteriospermia is defined as the presence of bacteria in seminal fluid samples causing (8). abnormal fertility process through the following mechanisms: deterioration of spermatogenesis, decreased sperm motility, altered acrosome reaction, altered morphology, formation of reactive oxygen species leading to increased DNA fragmentation index, formation of antisperm antibodies due to breach in the blood –testes barrier, and genital tract obstruction due to inflammation and fibrosis (9).

Enterobacteriaceae is associated with epididymitis, orchitis and prostatitis, suggesting that they may have a role in infertility. There is evidence that Gram positive cocci

include *Enterococci*, *Streptococci* and *Staphylococci* are associated with Prostatitis and epididymitis as well (10).

The impact of infection on infertility has been the subject of controversy since 1970s, and several treatment trials have been initiated since then. The criteria for infection-associated infertility have been laid down in the World Health Organization (WHO) manuals(1).

Aim of this study is to investigate the semen culture infection and its impact on semen parameters among infertile men.

Subject, materials and methods

1. Specimens collection:

This study was conducted at High Institute for Infertility Diagnosis

Assisted Reproductive Technology in Baghdad /al Kadhmiya for the period from June to July 2019. Fifty samples were collected in sterile cases from men who attended the Higher Institute of Infertility and Assisted Reproductive Techniques. Semen samples were collected by masturbation into wide-mouth glass or plastic containers, supplied by the laboratory, after 3–7 days of sexual abstinence. The sample was transported to the laboratory immediately and placed in an incubator at 37°C till complete liquefaction. Semen samples were analyzed by a macroscopic and microscopic examination as shown in Table (1) using standardization according to WHO standard (1).

Table (1): Normal values of human semen (WHO, 2010)

Parameters	Normal Values Analysis
Liquefaction	Complete within 60 minutes at room temperature.
Appearance	Homogeneous, gray and opaque.
Consistency	Leaves a pipette as discrete droplets.
Volume	1.5ml- 6ml.
PH	7.2-8.0
Concentration	15 million sperm / ml semen or greater.
Active Motility	32% or more with progressive motility and 40 % or more total motility (progressive and non-progressive)
Morphologically Normal Sperm	30% or more with normal forms by staining.
Viability	58% or greater.
Round Cells	<5/HPF

The involved parameters are the following

Appearance:

The semen is considered normal when the appearance is gray. It may appear less opaque if the sperm concentration is very low(1)

Volume:

The volume of the ejaculate was measured by using a graduated cylinder

with a conical base. Sample considered hypervolemic when the volume more than 6 ml and considered hypovolemic when the volume is less than 1.5 ml (1).

Liquefaction:

Normal semen sample liquefies within 60 minutes at room temperature. The incomplete mixing is probably a major contributor to errors in the determining of the sperm concentration (1).

Viscosity:

The viscosity of the semen sample was estimated by gentle aspiration into Pasteur pipette. The sample is considered normal when the semen leaves the pipette as small drop by drop. If the drops are not formed and the semen cannot easily draw up into pipette, this indicates a high viscosity (11).

pH:

The pH of the semen was measured by using pH litmus paper (range from 6-14). The pH of the semen was considered to be normal when it is slightly alkaline and ranges between (7.2-8.0).

Microscopic Examinations:

For each sample, a drop (10 μ l) of liquefied, thoroughly mixed semen was placed on a warm slide and covered with standard cover slip (22 X 22 mm). The preparation was scored under 40X objective.

Sperm Concentration:

Sperm concentration was measured from the mean number of sperm in five high power fields under magnification of 400 x .The lower reference limit for sperm concentration is 15×10^6 spermatozoa per mL and lower reference limit for the total sperm number is 39×10^6 spermatozoa per ejaculate(1).

Sperm Motility:

The number of motile sperm is examined in five randomly selected fields was counted away from the cover

slip edge. At least one hundred spermatozoa were counted.

Sperm Morphology:

The examination of morphologically normal sperm (MNS) was performed by using the same prepared slides for sperm motility, At least 100 spermatozoa were counted and the percentage of morphologically normal sperm was calculated according standard formula (1).

Sperm Agglutination:

Agglutination of spermatozoa means the motile spermatozoa stick to each other head-to-head, tail-to-tail or may in a mixing way. e.g., head to tail. This should be identified are record the adherence may be either of immotile spermatozoa to each other or motile spermatozoa to mucous threads. Cells other than spermatozoa or debris is considered nonspecific aggregation rather than agglutination and that should be recorded (1).

Round Cells Concentration:

The round cells in each human semen sample were considered as leukocytes and other cells such as spermatocyte, epithelial cell, the prostatic cell and others. The number of round cells were counted using High Power Field HPF methods (1).

4. Identification of bacterial isolates:

The primary identification based on morphological characteristics bacterial colonies growth on the selective media blood agar, macconkey agar and Hi crome UTI selective agar for 24 hrs. at 37 °C.

5. Statistical Analysis:

The Statistical Analysis System-SAS (12) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) or T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability in this study.

Results and Discussion

Frequency of Bacterial Isolates:

Among the total (50) samples, 45 (90 %) were infected with at least one type of the following bacteria . *E.coli*, *Klebsiella* spp., *Enterococcus* spp., *Staphylococcus* spp. and *Proteus* spp. Five samples of infertile semen were failed to detect microorganisms (Table

2), which is maybe due to either the samples were lacking of bacteria or the media used were insufficient to culture these organisms.

Bacterial invasion of male reproductive tract have a harmful effect on spermatozoa and play role in diminishing sperm quality through colonizing and contaminating the male urogenital tract this issue may raises the most controversy(13).

Microorganisms can affect the male reproductive function either directly, causing agglutination of motile sperm and thus reducing the ability of acrosome reactions and making alterations in cell morphology, or indirectly through production of reactive oxygen species generated by the inflammatory response to infection(14,15).

Table (2): Relative frequency of number of isolated bacteria among infertile patients.

Infertile patients	No	Percentage (%)
Negative	5	10
One isolate	12	24
Two isolate	33	66
Total	50	100%
P-value	---	0.0001 **

** (P<0.01).

Many studies have examined the impact of genital tract infections and bacterial semen contamination in male fertility, however, the putative detrimental effect of bacteria on the sperm quality is still controversial (15). There are direct and indirect effects of bacteria on the reproduction function in male, direct effect may include during bacterial semen infection, sperm motility and normal morphology loss may be consequences of adhesion phenomena and sperm agglutination. The sperm surface is rich in glycoproteins and is thus susceptible to bacteria– spermatozoa interactions at

the receptor–ligand level (15) while the indirect effect of bacteria on reproductive function in male may contributed to the role of reactive oxygen species (ROS) in sperm cell biology. At low levels, ROS play a physiologically important role in sperm hyperactivation, capacitation, and acrosome reaction; at higher levels, they cause oxidative stress that limits the fertilizing potential of the male gametes as a result of peroxidative damage to cellular macromolecules (13). Previous study reported the isolation of nine species of bacteria belonging to five genera, *Staphylococcus*, *Escherichia*,

Streptococcus, *Enterococcus* and *Klebsiella*, from semen (16). Study that the bacteriospermia may be direct causes of subfertility or additional negative factors worsening the prognosis of fertility in natural and assisted procreation (17). According to studies, *Staphylococcus aureus* and *Escherichia coli* were the highest detected bacteria from semen (18). One study indicated that overall bacterial content of sperm might not play a major role in male infertility (19).

In another hand, comparison between *Escherichia coli* and *Clostridium perfringens* on boar spermatozoa revealed that *E. coli* has a greater capacity to adhere to the sperm surface than *C. perfringens* during liquid storage at 17 °C, but damage on sperm membrane integrity induced

by *C. perfringens* occurred in a shorter period of time (20).

The effect of growth on seminal Parameters

The results as show in Table (3) showed there are statistical significance in semen volume in no growth (3.25 ± 0.87) and growth (2.45 ± 0.68) 0.662, and PH in no growth (8.51 ± 0.54) and growth (7.87 ± 0.49) 0.572. There are no statistical significance effect of growth and no growth on other Parameters, the result of the present study are compatible with WHO (1).

The presence of bacteria in semen samples of infertile men has a similar prevalence to that observed in fertile males (15). The clinical significance of bacteria in semen is still unclear.

Table (3): Comparison between no growth and growth in parameters

Parameters	Mean \pm SD		T-test
	-	+	
Semen volume (ml)	3.25 ± 0.87	2.45 ± 0.68	0.662*
Liquefaction time (minutes)	40.00 ± 13.14	35.91 ± 8.05	11.436 NS
PH	8.51 ± 0.54	7.87 ± 0.49	0.572*
Sperm concentration (million/ml)	38.08 ± 21.24	34.84 ± 18.63	15.409 NS
Normal morphology	35.42 ± 10.96	30.68 ± 6.31	8.917 NS
Agglutination	1.25 ± 4.33	2.29 ± 0.96	1.153 NS
Round cell count	7.96 ± 6.15	13.35 ± 7.02	8.612 NS

* (P<0.05),z NS: Non-Significant.

The volume, liquefaction time, pH, concentration, morphology, agglutination and round cell count for semen without the presence of bacteria were 3.25/ml, 40 min, 8.5, 38.08 million/ml, 35.42, 1.25 and 7.96, respectively (Table 2). While, the seminal parameters in the existence of bacteria were 2.4/ml, 35.9 min, 7.87, 34.84 million/ml, 30.68, 2.29, 13.35 for volume, liquefaction time, pH, concentration, morphology,

agglutination and round cell count, respectively (Table 2).

However, the latest World Health Organization (WHO) reference values for human semen characteristics were: semen volume 1.5 ml; total sperm number 39 million per ejaculate; sperm concentration 15 million per ml; vitality 58% live; progressive motility 32%; total motility 40%; morphologically normal forms 4.0%. Semen quality of the reference population was superior to that of the men from the general

population and Normozoospermic men (21).

The 'WHO manual for the examination of human semen and sperm [semen]-cervical mucus interaction' (22, 23, 11) is widely used as a source of standard methodology for laboratories engaged in semen analyses. High percentage of fertile men would be classified as subnormal, especially when morphology, sperm concentration or motility is considered (24, 25, 26). On the other hand, a sperm concentration of 20 million/ml, the 'normal' or 'reference' value cited by WHO (27,28,11), has been considered too low for a lower reference limit because the probability of pregnancy is essentially linear with sperm concentrations up to 40-50 million /ml (29, 30).

Relationship between bacterial isolates and seminal parameters

1. Semen volume

The results in Table (4) showed comparison of semen volume according to the results of bacteriological culture, it had been reported that the highest semen volume (2.73 ± 0.7) found *Enterococcus* infected patients. In the present study, the lowest seminal volume were reported in the presence of *E. coli* (1.83 ± 0.93) and *Klebsiella* (2.35 ± 0.82) with significant p value for *E. coli* ($p=0.004$) and *Klebsiella* ($p=0.048$) compared with uninfected patients (Table 4) however, is significantly low, According to WHO .while, no significant differences (p value ≥ 0.05) found in seminal volume infected with following genera *Enterococcus*, *Staphylococcus* and *Proteus* (Table 4).

Table (4): Effect of bacterial isolates on semen volume.

Bacterial isolates	Mean \pm SD of Semen volume (ml)
<i>E. coli</i>	1.83 ± 0.93 b
<i>Klebsiella</i>	2.35 ± 0.82 ab
<i>Enterococcus</i>	2.73 ± 0.7 a
<i>Staphylococcus</i>	2.79 ± 0.91 a
<i>Proteus</i>	2.57 ± 0.79 ab
LSD value	0.802 *

Means having with the different letters in column differed significantly, * ($P<0.05$).

2. Liquefaction time.

The results in Table (5) showed comparison in liquefaction time according to bacterial culture, there were none statistical difference in the mean of each groups *E.coli* (34.17 ± 7.36), *Klebsiella* (35 ± 5.77), *Enterococcus* positive group (36.85 ± 8.76), *Staphylococcus* group (33.57 ± 6.9) and *Proteus* infected group (40 ± 11.9) were none significantly different, According to WHO. It has been demonstrated by (Kumar and Garg,2019) that *E. coli* coming into contact with spermatozoa causes

decreased sperm motility .The decrease in sperm motility due to *E. coli* has been attributed to an agglutinating effect on sperm that sperm agglutination can be caused by bacterial type 1 and P fimbriae; specifically, the type 1 fimbriae of *E. coli* cause a pattern of head-head type agglutination because they bind mannose residues in the head region of sperm. Instead, type P fimbriae of *E. coli* cause a head-tail agglutination pattern because they bind gal-gal receptors present along the sperm (32).

Table (5): Effect of bacterial isolates on liquefaction time.

Bacterial isolates	Mean \pm SD of Liquefaction time (minutes)
<i>E. coli</i>	34.17 \pm 7.36
<i>Klebsiella</i>	35.00 \pm 5.77
<i>Enterococcus</i>	36.85 \pm 8.76
<i>Staphylococcus</i>	33.57 \pm 6.90
<i>Proteus</i>	40.00 \pm 11.90
LSD value	13.094 NS

NS: Non-Significant.

No statistical differences (p value \geq 0.05) were found between bacterial isolates and liquefaction time (Table 5).

3. PH.

The results in Table (6) showed comparison in pH according to bacterial culture, there were statistical reduction in the mean of pH in each groups *E. coli* (7.98 \pm 0.31), *Klebsiella* (7.92 \pm 0.24), *Enterococcus* positive group (7.84 \pm 0.18), *Staphylococcus* group

(7.89 \pm 0.27) and *Proteus* infected group (7.73 \pm 0.34) were significantly different, According to WHO. However, the importance of semen pH in fertility prognosis of these patients is not clear. From a diagnostic point of view semen pH cannot be recommended as a tool to discriminate infected from non-infected patients, due to its low sensitivity and specificity(15).

Table (6): Effect of bacterial isolates on pH.

Bacterial isolates	Mean \pm SD of pH.
<i>E. coli</i>	7.98 \pm 0.31
<i>Klebsiella</i>	7.92 \pm 0.24
<i>Enterococcus</i>	7.84 \pm 0.18
<i>Staphylococcus</i>	7.89 \pm 0.27
<i>Proteus</i>	7.73 \pm 0.34
LSD value	0.588 NS

NS: Non-Significant.

The pH of the infected sperm were dropped down compared with negative group with significant differences p value \leq 0.05 (Table 6). The reason for that decrease is because of the presence of the microorganisms. The study is in accordance with previous report exhibited the pH of the semen of 10 patients dropped into <7.2 by presence of bacteria (18).

4. Sperm concentration:

The results in Table (7) showed comparison in sperm concentration

according to bacterial culture, there were statistical reduction in the mean of sperm concentration (million/ ml) in each groups *Staphylococcus* (44.43 \pm 25.24), *E.coli* (40.67 \pm 21.5), *Enterococcus* positive group (35.26 \pm 29.29) as the same with *E. coli* and group *Klebsiella* (23.44 \pm 23.01) *Proteus* infected group (30.43 \pm 16.8) were none significantly different, According to WHO.

Table (7): Role of bacterial isolates on sperm concentration.

Bacterial isolates	Mean \pm SD of Sperm concentration (million/ml)
<i>E. coli</i>	40.67 \pm 21.5 ab
<i>Klebsiella</i>	23.44 \pm 23.01 b
<i>Enterococcus</i>	35.26 \pm 29.29 ab
<i>Staphylococcus</i>	44.43 \pm 25.24 a
<i>Proteus</i>	30.43 \pm 16.8 ab
LSD value	19.055 *

Means having with the different letters in column differed significantly, * (P<0.05).

Our observation in this report revealed that the sperm concentrations were reduced in the presence of *Klebsiella* spp, and *Enterococcus* spp, while concentration found to be higher in the presence of *E. coli*, *Staphylococcus* and *Proteus* compared with negative group (Table 7). Significant differences (p value 0.001) were found in the presence of *Proteus*. it has been demonstrated that the sperm concentration of Normozoospermic candidates with bacteria presence although had normal range of sperm concentration ($>20 \times 10^6/ml$), but had always been lower compared with those without bacterial presence (18). Several investigations that assessed *in vitro* fertilization indicated that oocyte fertilization was reduced in the presence of pathogenic organisms in semen (31) and concluded that semen bacteria

contamination reduces semen quality, interferes with fertilization. Increased prevalence of genital tract infections caused by *E. faecalis* was associated with compromised semen quality in terms of sperm concentration and morphology (32).

5. Abnormal morphology:

The results in Table (8) showed comparison in mean percentage of normal sperm morphology according to bacterial culture, there were statistical reduction in *E. coli* infected group (18.83 \pm 19.59) and *Staphylococcus* (28.57 \pm 13.76) while, other groups like: *Klebsiella* (35.8 \pm 9.3), *Enterococcus* positive group (35.92 \pm 11.34), and *Proteus* infected group (34.29 \pm 8.99) were none statistically significant different from, according to WHO.

Table (8): Role of bacterial isolates on abnormal morphology.

Bacterial isolates	Mean \pm SD of Normal morphology
<i>E. coli</i>	18.83 \pm 19.59 b
<i>Klebsiella</i>	35.80 \pm 9.30 a
<i>Enterococcus</i>	35.92 \pm 11.34 a
<i>Staphylococcus</i>	28.57 \pm 13.76 ab
<i>Proteus</i>	34.29 \pm 8.99 a
LSD value	11.058 *

Means having with the different letters in column differed significantly, * (P<0.05).

Abnormal morphology of sperm were found in patients infected with *E. coli* that shows statistical significant (p value 0.035). No significance differences (p value >0.05) observed in the sperm morphology with patients infected with *Klebsiella* spp,

Staphylococcus spp, *Enterococcus* spp and *proteus* spp (Table 9).

6. Agglutination:

The results in Table (9) showed comparison in mean of agglutination according to bacterial culture, there were statistically significant in *Proteus* (4.29 \pm 2.35) and *Enterococcus* (4.23 \pm

2.72) infected group, and none different from, according to WHO. statistically significant in other isolated

Table (9): Role of bacterial isolates on agglutination.

Bacterial isolates	Mean \pm SD of Agglutination
<i>E. coli</i>	0.00 \pm 0.00 b
<i>Klebsiella</i>	1.50 \pm 2.74 ab
<i>Enterococcus</i>	4.23 \pm 2.72 a
<i>Staphylococcus</i>	1.43 \pm 1.78 ab
<i>Proteus</i>	4.29 \pm 2.35 a
LSD value	3.261 *

Means having with the different letters in column differed significantly, * (P<0.05).

Moreover, No significance differences (p value >0.05) observed in the sperm agglutination with patients infected with all the tested bacteria (Table 9). Microorganisms might affect the male reproductive function causing the alterations in cell morphology, reducing ability for the acrosome reaction and also causing the agglutination of motile sperm. In another report reported the agglutination of spermatozoa due to adhesion to *E. coli* leading to morphological alterations

in sperm involving plasma membrane and degeneration of acrosome (33, 34).

7. Round cell

The results in Table (10) showed comparison in mean of round cell according to bacterial culture, there were statistically significant in *Staphylococcus* (15.57 \pm 8.94), and none statistically significant in other isolated different from , according to WHO.

Table (10): Role of bacterial isolates on round cell count.

Bacterial isolates	Mean \pm SD of Round cell count
<i>E. coli</i>	15.58 \pm 8.38 a
<i>Klebsiella</i>	7.65 \pm 4.02 b
<i>Enterococcus</i>	11.46 \pm 5.29 ab
<i>Staphylococcus</i>	15.57 \pm 8.94 a
<i>Proteus</i>	11.50 \pm 7.25 ab
LSD value	6.933 *

Means having with the different letters in column differed significantly, * (P<0.05).

8. Sperm motility:

The result in Table (11) showed comparison in sperm grade according to bacterial culture, there were statically significant reduction in the men of grade D in *E. coli* (67.83 \pm 32.34),

Staphylococcus (47.14 \pm 22.44), *Klebsiella* (40.0 \pm 11.06) *Proteus* (37.86 \pm 8.09) and *Enterococcus* (36.31 \pm 7.39). There were none statically significant reduction in the men's of other grades.

Table (11): Role of bacterial isolates on sperm motility.

Bacterial isolates	Grade A	Grade B	Grade C	Grade D
<i>E. coli</i>	7.38 \pm 12.2 b	16.83 \pm 24.24 b	15.33 \pm 9.93 b	67.83 \pm 32.34 a
<i>Klebsiella</i>	5.93 \pm 2.27 b	31.0 \pm 11.01 a	29.0 \pm 5.68 a	40.0 \pm 11.06 b
<i>Enterococcus</i>	23.25 \pm 12.2 a	35.62 \pm 9.82 a	28.08 \pm 7.51 a	36.31 \pm 7.39 b
<i>Staphylococcus</i>	23.25 \pm 12.2 a	28.0 \pm 17.12 a	25.71 \pm 10.48 a	47.14 \pm 22.44 b
<i>Proteus</i>	23.25 \pm 12.2 a	35.71 \pm 9.32 a	26.43 \pm 8.52 a	37.86 \pm 8.09 b
LSD value	6.548 **	8.019 **	5.953 **	13.602 **

Means having with the different letters in column differed significantly, ** (P<0.01).

The motility of the sperm in the presence of *E. coli* showed general reduction in grade A, B and C while, in grade D demonstrated higher motility. Significant differences (p value ≤ 0.05) were demonstrated in grade A and D compared with negative group (Table 11). The present study is relevant to previous study stated the sperm motility was significantly lower in *E. coli* contaminated samples than in the control group, and the presence of *E. coli* and *S. aureus* led to a decline in normal morphology of the sperms (17). In particular, *E. coli* strains are known for their ability to immobilize and damage the morphology of spermatozoa by direct contact, mediated by attachment organelles such as pili or type-1 fimbriae and mannose receptor-dependent interactions (35).

Meanwhile, the sperm motility in the presence of *Klebsiella* sp, showed decline in grade A motility with p value 0.001. Furthermore, no significant

differences ($p > 0.05$) were found in other grades. No statistical differences ($p > 0.05$) in sperm motility were found in the presence of *Staphylococcus*, *Enterococcus* and *Proteus* (Table 11). Slow progressive motility were found commonly with gram positive organisms as a major factor contributory to poor sperm quality among this group, followed by abnormal morphology (32,18).

9. Role of number of isolated bacterial on seminal fluid parameter.

The result in Table (12) showed comparison of semen parameter according to number of isolate bacteria, anova test showed that there was statically significantly in semen concentration whom infected with one isolate (40.06 ± 22.29) or tow isolate (13.88 ± 17.87). None statically significantly in all other parameters, according to WHO.

Table (12): Role of number of isolated bacterial on seminal fluid analysis.

Seminal fluid analysis parameters	One isolates	Two isolates	T-test
Semen volume (ml)	2.53 ± 0.86	2.40 ± 0.82	0.429 NS
Liquefaction time (minutes)	35.73 ± 8.75	37.00 ± 6.71	5.934 NS
PH	7.85 ± 0.29	7.92 ± 0.11	0.498 NS
Concentration (million/ml)	40.06 ± 22.29	13.88 ± 17.87	18.563 *
Grade A	10.28 ± 8.3	8.19 ± 3.03	4.528 NS
Grade B	9.39 ± 7.53	7.00 ± 6.25	6.477 NS
Grade C	24.30 ± 9.02	31.00 ± 7.42	8.025 NS
Grade D	40.00 ± 8.94	46.48 ± 20.33	16.247 NS
Normal morphology	29.33 ± 13.55	41.00 ± 7.42	15.732 NS
Sperm agglutination	1.82 ± 4.48	5.00 ± 4.07	4.923 NS
Round cell count	13.05 ± 7.08	17.80 ± 4.62	6.051 NS

* ($P < 0.05$), NS: Non-Significant.

Different parameters of semen were tested against the presence of bacterial infection. No significant differences were observed in all the parameters in respect to number of isolated bacteria (Table 12). Negative relationship between the bacterial infections and

sperm parameters ($p < .010$), such as concentration, motility and progressive motility, were reported (16).

Many researches have worked on the urogenital tract specific and facultative bacterial contamination in male infertility; however, the putative

effect of these agents on the quality of semen is still controversial (36).

Further literature explanations resides on the existence of an antigenic mimicry between some constituent of sperm flagella such as tubulin found in axoneme, and bacterial proteins which may have pathogenic effect (37). Infection may therefore induce antibodies and T-cells to react against bacterial cell constituents that may recognize self-components and immune mediated damage may follow. But simply, spermatozoa may share epitopes with bacteria of the most frequent species colonizing the genitourinary tract of man. The antigen may induce an antibody response that could cross-react with the flagella of spermatozoa affecting its life span and motility (38).

Infectious factors trigger the infiltration of leukocytes to the inflammatory site. Leukocytospermia is generally attributed to the inflammation or infection of semen. According to the kinetics of the inflammatory process in the urogenital tract, leukocytes appear in semen as the addition to bacteriospermia at the second stage of the urogenital tract infection, and remain present in semen for some length of time following the elimination of the bacteria in the third stage (isolated leukocytospermia), There is an ongoing controversy concerning the biological role of the leukocytes attracted into the semen (13).

References

1. World Health Organization. WHO. (2010). Laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization.
2. Simon, L.; Brunborg, G.; Stevenson, M.; Luttor, D.; McManus, J. and Lewis, S.E.M. (2010). Clinical significance of sperm DNA damage in assisted reproduction outcome. *Hum Reprod.* 25(7): 1594-1608.
3. Cassuto, N.G.; Hazout, A.; Hammoud, I.; Balet, R.; Bouret, D.; Barak, Y. *et al.* (2012). Correlation between DNA defect and sperm-head morphology. *Report Biomed Online.*, 24(2): 211-218.
4. Francavilla, F.; Sciarretta, F.; Sorgentone, S.; Necozone, S.; Santucci, R.; Barbonetti, A., *et al.* (2009). Intrauterine insemination with or without mild ovarian stimulation in couples with male subfertility due to oligo/astheno-and/or teratozoospermia or antisperm antibodies: a prospective cross-over trial. *Fertil Steril.*, 92(3): 1009 -1011.
5. Ashok, A.; Aditi, M.; Alaa, H. and Michelle, R. (2015). A unique view on male infertility around the globe. *Reprod Biol Endocrinol.*; 13:37-41.
6. Askienazy-Elbhar, M. (2005). Male genital tract infection: the point of view of the bacteriologist. *Gynecol Obstet Fertil.*; 33(9):691-697.
7. Khadhim, M.F. and Abdul-Hassan, I.A. (2017). Association of Androgen Receptor Gene Polymorphisms at three SNPs and their Haplotypes with Severe Oligozoospermia Risk in Iraqi Patients. *Iraqi Journal of Biotechnology*, 16, (1): 16-28.
8. Liu, J.H.; Li, H.Y.; Cao, Z.G.; Duan, Y.F.; Li, Y. and Ye, Z.Q. (2002). Influence of several uropathogenic microorganisms on human sperm motility parameters in vitro. *Asian J Androl.*; 4(3):179- 182.
9. Vilvanathan, S.; Kandasamy, B.; Jayachandran, A.L.; Sathiyarayanan, S.; Singaravelu, V.T.; Krishnamurthy, V., *et al.* (2016). Bacteriospermia and Its Impact on Basic Semen Parameters among Infertile Men. *Interdisciplinary Perspectives on Infectious Diseases*, Volume, vol: 1-6.
10. Pellati, D.; Mylonakis, I.; Bertoloni, G.; Fiore, C.; Andrisani, A.; Ambrosini, G., *et al.* (2008). Genital tract infections and infertility. *Eur J. Obstet Gynecol Reprod Biol.*; 140:3-11.
11. Silverberg, K.M. and Turner, T. (2001). Evaluation of sperm. In: *TextBook of Assisted Reproductive Techniques Laboratory and Clinical Perspectives*. D. K. Gardner, A.; Weissan, C. M. Howles, and Z. Shonam (eds), Martin DunitLtd, The livert House, 7-9 pratt street, London, UK. P p: 61-76.

12. SAS (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
13. Fraczek, M. and Kurpisz, M. (2015) Mechanisms of the harmful effects of bacterial semen infection on ejaculated human spermatozoa: potential inflammatory markers in semen. *Folia Histochem Cytobiol.*; 53(3): 201-217.
14. Tremellen, K. (2008). Oxidative stress and male infertility-- a clinical perspective. *Hum Reprod Update*; 14:243-258.
15. Moretti, E.; Capitani, S.; Figura, N.; Pammolli, A.; Federico, M.G.; Giannerini, V., *et al.* (2009). The presence of bacteria species in semen and sperm quality. *J Assist Reprod Genet*; 56-47 :1)26.
16. Zeyad, A.; Hamad, M.F. and Hammadeh, M.E. (2018). The effects of bacterial infection on human sperm nuclear protamine P1/P2 ratio and DNA integrity. *Andrologia*. Mar; 50(2):e12841.
17. Enwurua, C.A.; Iwalokuna, B.; Enwuru, V.N.; Ezechi, O. and Oluwadun, A. (2016). The effect of presence of facultative bacteria species on semen and sperm quality of men seeking fertility care. *African journal of urology*, 22(3): 213-222.
18. Baud, O.; Trousson, C.; Biran, V.; Leroy, E.; Mohamed, D. and Alberti, C. (2019). Two-year neurodevelopmental outcomes of extremely preterm infants treated with early hydrocortisone: treatment effect according to gestational age at birth. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, 104(1): F30-F35.
19. Bonet, S.; Delgado-Bermúdez, A.; Yeste, M. and Pinart, E. (2018). Study of boar sperm interaction with *Escherichia coli* and *Clostridium perfringens* in refrigerated semen. *Animal reproduction science*. 197: 134-44.
20. Cooper, T.G.; Noonan, E.; Von Eckardstein, S.; Auger, J.; Baker, H.W.; Behre, H.M., *et al.* (2010). World Health Organization reference values for human semen characteristics. *Human reproduction update*. Jan 1; 16(3): 231-45.
21. World Health Organization. WHO. (1987). *Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction*, 2nd edn. Cambridge: Cambridge University Press, , 80 p.
22. World Health Organization. WHO. (1992). *Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction*, 3rd edn. Cambridge: Cambridge University Press, , 107 p.
23. Nallella, K.P.; Sharma, R.K.; Aziz, N. and Agarwal, A. (2006). Significance of sperm characteristics in the evaluation of male infertility. *Fertil Steril*; 85(3): 629-634.
24. Pasqualotto, F.F.; Sobreiro, B.P.; Hallak, J.; Athayde, K.S.; Pasqualotto, E.B. and Lucon, A.M. (2006). High percentage of abnormal semen parameters in a prevasectomy population. *Fertil Steril*; 85:954-960.
25. Gao, J.; Gao, E.S.; Walker, M.; Yang, Q.; Wu, J.Q.; Zhu, Q.X., *et al.* (2008). Reference values of semen parameters for healthy Chinese men. *Urol Int*; 81(3): 256-262.
26. Bostofte, E.; Serup, J. and Rebbe, H.; (1983). Has the fertility of Danish men declined through the years in terms of semen quality? A comparison of semen qualities between 1952 and 1972. *Int J. Fertil*; 28(2): 91-95.
27. Lemcke, B.; Behre, H.M. and Nieschlag, E. (1997). Frequently subnormal semen profiles of normal volunteers recruited over 17 years. *Int J Androl*; 20(30): 144-152.
28. Bonde, J.P.; Ernst, E.; Jensen, T.K.; Hjollund, N.H.; Kolstad, H.; Henriksen, T.B., *et al.* (1998). Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet*; 352(9135): 1172-1177.
29. Slama, R.; Eustache, F.; Ducot, B.; Jensen, T.K.; Jørgensen, N.; Horte, A., *et al.* (2002). Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. *Hum Reprod*; 17:503-515.
30. Almond, G.W. and Poolperm, P. (1996). Semen contamination and choosing antibiotics. In: *Proceedings of the North Carolina healthy hogs seminar...* p. 17-20.
31. Qiang, H.; Jiang, M.S.; Lin, J.Y. and He, W.M. (2007). Influence of enterococci on human sperm membrane *in vitro*. *Asian J. Androl*; 9(1): 77-81.
32. Kumar, V. and Garg, N. (2019). Effect of *Escherichia coli* on Semen Quality of Infertile Human Male. *Virol Immunol J.*, 3(3): 000214.
33. Solomon, M. and Henkel, R. (2017). Semen culture and the assessment of genitourinary tract infections. *Indian J. Urol.*, 33(3): 188-193.
34. Agarwal, J.; Srivastava, S. and Singh, M. (2012). Pathogenomics of uropathogenic

- Escherichia coli. Indian J Med Microbiol.; 30(2): 141–149.
35. Nabi, A.; Khalili, M.A.; Halvaei, I.; Ghasemzadeh, J. and Zare, E. (2013). Seminal bacterial contaminations: Probable factor in unexplained recurrent pregnancy loss. Iran J Reprod Med.; 11(11): 925-932.
 36. Moretti, E.; Capitani, S.; Figura, N.; Pammolli, A.; Federico, M.G.; Giannerini, V., *et al.* (2009). The presence of bacteria species in semen and sperm quality. J Assist Reprod Genet; 26(1): 47-56.
 37. Grassme, H.; Jendrossek, V. and Gulbins, E.; (2001). Molecular mechanisms of bacteria induced apoptosis. Apoptosis; 6(6): 441–445.
 38. AL-Haboubi, H. M. (2018). Molecular Study of Herpes Simplex Virus (1and2) and Methylation Pattern of MTHFR gene in Infertile Men. (MSc thesis). Institute of genetic engineering and biotechnology for post graduate studies / University of Baghdad.