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# Influence of Bacterial Infection on Human sperm

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## Abstract:

**Background:** The invasion of bacteria into the reproductive system of men correlates with diminished sperm functionality, resulting in damaged fertilization capacity. The human body contains trillions of microbes, and their effects on health have been investigated in various bodily systems. There is an agreement regarding the detrimental effects of certain pathogenic bacterial species on semen variables, involving sperm motility, count, morphology, and sperm DNA integrity. This investigation aimed to assess the effects of microbial infection in the male reproductive system on human sperm variables and functioning. This study comprised 186 semen samples from sub-fertile males and unselected couples seeking assistance at the infertility clinic. All semen samples underwent bacteriological examination, and both semen and sperm quality have been assessed following World Health Organization guidelines. **(WHO,2010).**

**Results:** Among the 186 cases examined for infertility, 65 (34.94 percent) of the analyzed semen samples have been contaminated with various bacterial species. The bacterial strains discovered were *Staphylococcus Haemolyticyus*, *Enterococcus Fecails*, *Micrococcus Lylae*, *Escherichia coli*, and *Serratia marcescens*. Infected semen in sub-fertile men adversely affects sperm quality, including motility, count, progression, vitality, and normality. Furthermore, elevated levels of reactive oxygen species (ROS) correlated with diminished sperm functionality, including acrosin activity (A.A) and hypo-osmotic swelling test (HOST) have been observed in infected sub-fertile males compared to their non-infected sub-fertile males, but the differences were statistically insignificant.

**Conclusion:** Infected semen adversely affects sperm parameters and function, resulting in diminished fertilization capability of sperm of humans.

**Keywords**: Infection, Sperm quality, HOST, ROS, and Acrosin activity

## Introduction:

Bacterial infection of the male genital system is associated with low sperm activity and cases of infertility (1). Male genital system inflammation is associated with about 15% of cases of male infertility. Sixty percent of cases treated with assisted reproduction technology (ART) had microbial infection or inflammation (2). It has been discovered that the presence of pathogenic microorganisms in semen specimens had negative effects on sperm parameters (3). The clinical data suggest that male urogenital tract infections may

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inhibit male infertility by affecting sperm directivity or indirectly by acting on the regulatory system (4), which inversely affects assisted reproductive techniques (5). The high sperm quality parameters and oocytes are very essential for the fertilization process achievement and embryo development. The spermatozoa bad quality leads to a low fertilization percentage (6).

Urogenital inflammation can affect the male reproductive system in numerous manners. Inflammation influences spermatogenesis & sperm functionality, either directly or indirectly. Through sperm antibodies, the production of reactive oxygen species (ROS) & DNA damage (7). Elevated levels of reactive oxygen species in seminal plasma are associated with lipid peroxidation of the sperm plasma membrane, potentially resulting in diminished sperm fertilization capacity (8). Urogenital system infections can arise from several reasons, including environmental pollutants, vaginal products throughout intercourse, consumption of alcohol, tobacco usage, specific medications, & operations (9). Urogenital system infection alters the composition of seminal plasma, therefore obstructing the genital tract. Furthermore, a breach of the blood testis barrier resulting from inflammation & infection leads to the production of anti-sperm antibodies, potentially limiting sperm fertilization ability (10,11).

Previous research regarding the impact of bacteria on human fertility is contradictory; for example, a semen analysis of 207 cases revealed bacterial presence in 167 men (80.7 percent). No adverse effects were observed on fertilization or pregnancy rates. Consequently, they are against any bacteriological assessment in couples with unexplained infertility. The opposite result has been observed in a study of 382 couples, revealing that while the fertilization rate and early embryonic development remained affected by bacteria, the pregnancy rate per cycle was significantly diminished in the presence of ejaculate-contaminating bacteria. The varying conclusions may result from the presence of several bacterial species in differing numbers, which generate a different broad of diseases (12-14). Nevertheless, the majority of scientists refute the notion that bacteria adversely affect spermatozoa during ART, with evidence of diminished fertilization rates, poor embryo development, heightened miscarriage rates, and fetal death. This investigation aimed to assess the impact of bacterial infection on human sperm parameters, including motility, count, vitality, progressive normality, & sperm function (Acrosin Activity, HOST, and ROS).

## Material and methods:

A cross-sectional investigation has been performed between January 2018 and November 2019. This work included 186 infertile couples due to male infertility. All participants have been attendees of the assisted reproduction technology unit at the International Islamic Center for Population Studies and Research (IICPSR). Al Azhar University, Cairo, Egypt.

Inclusion criteria:

- The age of males ranged between 28-45 years old.
- All patients have the same cause of infertility (male factor).
- No azoospermia.

All males subjected to:

### 1- Semen specimen collection and Examination:

The semen specimens have been collected under sterile conditions by masturbation following 2 – 5 abstinence days. Patients avoid taking any antibiotics one week before semen collection specimens. Semen analysis has been conducted after liquefaction within sixty minutes of collection to evaluate sperm motility, count, vitality, normality, & sperm function tests. The variables of human sperm have been assessed following World Health Organization criteria (15).

### 2- Microbiological studies:

2.1 .Bacterial culture: Each specimen was immediately inoculated on blood agar plates and

Mac-Conkey's media plates. All plates have been incubated aerobically at 37 degrees Celsius for twenty-four to forty-eight hours (16).

2.2. Isolation and Identification of Microbial Isolates: The isolation and identification of microbial opportunistic pathogens in patient semen were performed through three main conventional steps:

- **Morphological characteristics**: On Mac-Conky's medium, we can differentiate the enteric bacteria into two groups; the lactose fermenters (Coli forms) by their pink colonies and the non-lactose fermenters, whose colonies are pale (Proteus) (17).

- **Microscopic Gram stain investigation:**
- The film of each pure bacterial isolate was prepared for 24 h. bacterial culture and stained using crystal violet as a basic dye and safranin as a counter stain. Gram-stained films were microscopically examined to differentiate both Gram-negative and Gram-positive bacteria as well as the shape and arrangement of cells (18).

- **Biochemical tests:**
- Biochemical tests: Microscopic examinations and biochemical tests used for identification were performed according to Bergey's Manual of Determinative Bacteriology Identifications were accepted from either system if the likelihood of that identification was greater than or equal to 85% (19). Further identification was carried out through molecular 16S RNA.

**3- Sperm concentration & motility estimation:**

Sperm concentration & motility have been assessed utilizing the Makler counting chamber (Sefi-Medical Instruments Lts); five microliters of the liquefied semen sample have been introduced to the chamber. The cover glass is subsequently positioned over the semen. Examined on the microscopic stage with phase contrast optics at a magnification of 200x (20x objective multiplied by 10x ocular). The sperm count was determined by dividing the total sperm count in one hundred squares by 10 x 1 million/ml, and motility was assessed as follows:

Total Sperm Motility % = the total number of motile sperm divided by total sperm count x 100

Sperm progressive = total number of Spermatozoa moving in one direction divided by sperm concentration (15).

**4- Evaluation of sperm vitality:**

Eosin staining has been utilized for sperm vitality evaluation. Staining has been carried out by {dissolved 0.5 g of eosin in 100 ml of 0.9 % Nacl}.10 µl of semen sample after mixing and liquefaction on dry and clean glass microscopic slide to an equal volume of eosin stain, then mixed by pipette tip, swirling the sample on the slide, then coverslip and left for 30 seconds. Examined was performed by Phase-contrast optic {400} Magnification. Unstained head sperm signified {live sperm} and pink or red colors {dead sperm}. Calculation of the mean percentage of sperm vitality:

Percent vitality = (number sperm unstained) / (number sperm unstained) + (number sperm with pink color) x100.

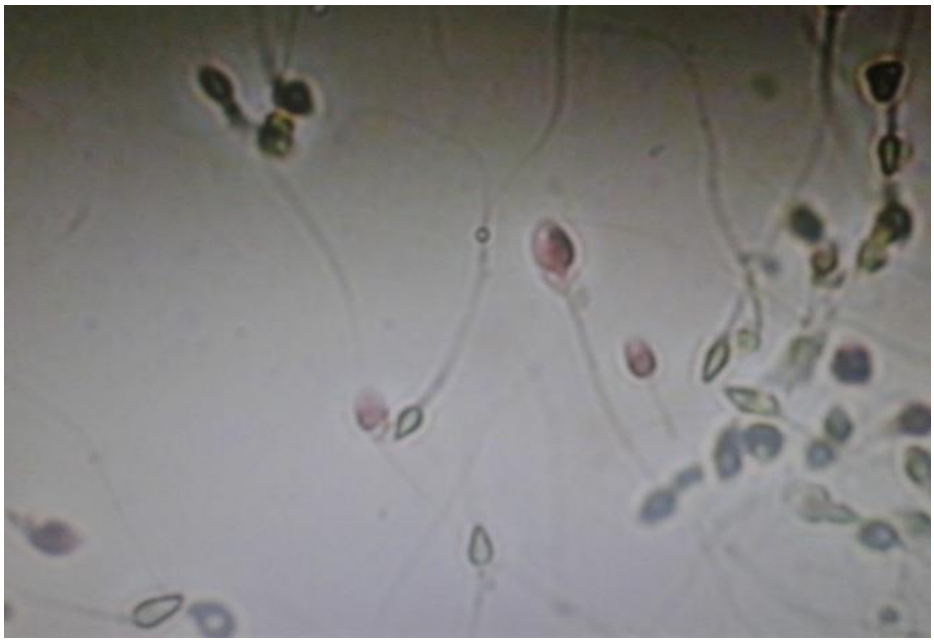

**Figure 1: Photograph of bright filed, high power field (HPF) (40-x), Representing sperm vitality:** Pink cells: non-vital (dead)/ Greenish cells: vital

## 5- Evaluation of sperm morphology:

To prepare a sperm smear for sperm morphology estimation, ten microliters of sperm suspension were transferred to clean a glass slide & allowed to air dry at room temperature, then the slide smear was stained in Diff-Quick stain. About 200 spermatozoa per slide have been examined under oil immersion with a magnification of 1000x (100x objective × 10x ocular) to distinguish between abnormal and normal spermatozoa according to (15). Morphologically abnormal spermatozoa frequently exhibit numerous abnormalities. In previous protocols. In the presence of many defects. Priority has been selected only to flaws of the sperm head over those of the midpiece, and to deficiencies of the midpiece over those of the tail.

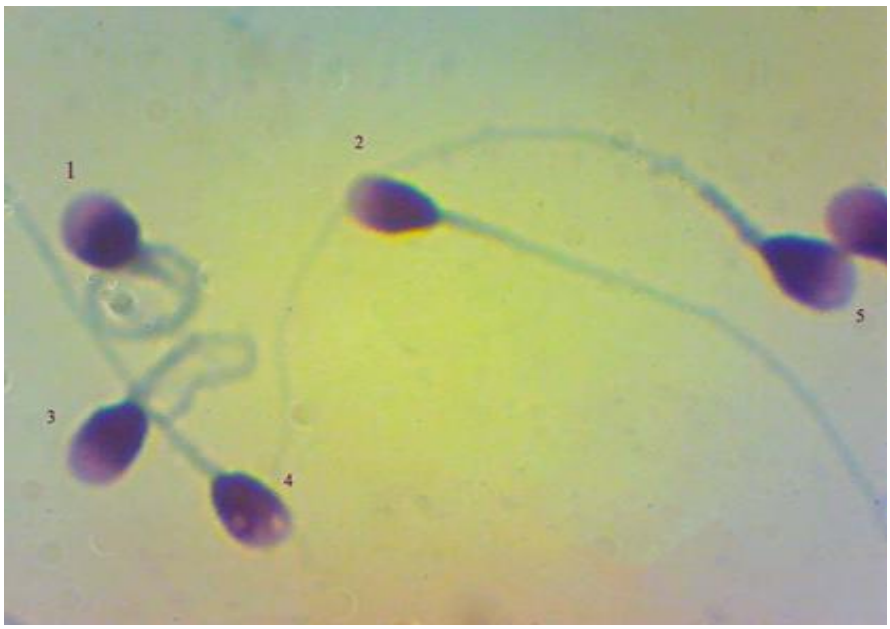


**Figure 2: Photograph of bright filed / HPF (100-x):** Represented sperm morphology Sperm No 2&5: normal in each (Head, Midpiece & tail). Sperm No.1: abnormal in each (Head, Midpiece &tail). Sperm No. 3: abnormal in the midpiece & tail), and Sperm No. 4: abnormal only in the Head.

### 6- Measurement of Reactive oxygen species (ROS) in semen by Malondialdehyde (MDA):

Malondialdehyde (MDA) levels in semen are an accepted measurement of oxidative stress. MDA was estimated by utilizing the method of thiobarbituric acid. Centrifuged the semen specimen after liquefying for seven minutes at two thousand grams, followed by taking one hundred microliters of supernatants, 0.9 ml of distilled water, and half a milliliter of thiobarbituric acid reagent{0.67 gram of 2-thiobarbituric acid mixed in one hundred milliliters of distilled water with half grams sodium hydroxide & added one hundred milliliters glacial acetic acid} to each tube has been added in boiling water for 1 hour and then cooling tubes under tap water, centrifuged all tubes for ten minutes at 400 g, & then calculate the absorbance of supernatant by spectrophotometer at 534 nm (20).

### 7- Assessment of Acrosin activity (A.A) in human sperm:

Acrosin, a sperm-specific acrosomal proteinase, plays an essential function in fertilization. Reduced concentrations of Acrosin are related to infertility and subfertility, and the Acrosin activity in spermatozoa may serve as a useful predictor of semen quality. This test has been conducted following (21).

### 8- Evaluation of Hypo-Osmotic Swelling Test (HOST):

The hypo-osmotic swelling (HOS) test has been done to assess sperm membrane integrity (sperm vitality) and was described as a test for sperm function. This test was performed according to (15, 22), Add 10 µl of the liquefied semen sample to 1 ml of HOS Solution (dissolved 0.735 g sodium citrate dehydrate and 1.351 g fructose mix in one hundred milliliters of distilled water), incubated at 37 degrees Celsius for 30 to 60 minutes. The transferred 10 µl of sperm suspension was placed on a microscopic slide and a cover slip. A total of two hundred spermatozoa were examined by a phase contrast microscope at magnification 400x (40x objective X 10x ocular) and the tail swelling sperm was observed.

Calculation of the mean percentage of swollen tail sperm:

$$\text{Percent swelling} = \frac{\text{Number of sperm with swollen tail}}{\text{Number of spermatozoa with swollen} + \text{non-swollen tails}} \times 100$$

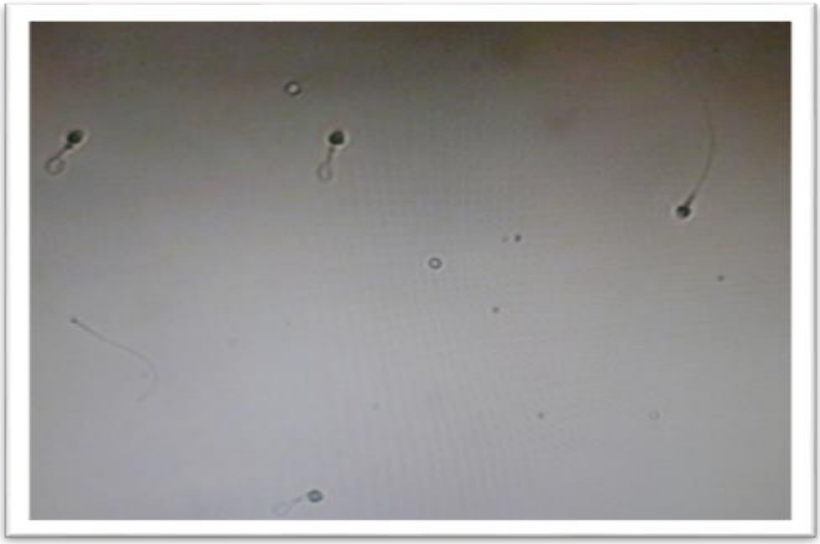

**Figure 3: Photograph by HPF.Represented the membrane integrity of human sperm**

1-Swelling tail (live) cells

2-Non-swelling tail (dead) cells

**Statistical analysis:** Data have been encoded & input utilizing the Statistical Package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). The student's t-test has been utilized to compare means across several groups. Fisher's exact test has been utilized to compare percentage values, with significance defined as $P \leq 0.05$.

**Results:**

Only 186 male patients have been involved in the investigation among 200 male patients who fulfilled the inclusion criteria. After semen culture results, they were divided into 2 groups: Infected semen sub-fertile men group: males partner with infected semen, n = 65 **cases;** non-infected semen sub-fertile men group: males partner with non-infected semen, n = 121 **cases**.

The results showed the distribution of groups regarding the results of bacterial semen cultures. The incidences of infected semen samples were 65 (34.94%), and the non-infected semen sample was 121 (65.06%) semen samples (table 1, figure 4).

The results showed a distribution of bacterial strains isolated from semen cultures. The indices of Gram-Positive bacteria were Enterococcus Facials strain 25 (38.46%), *Staphylococcus Haemolyticus* 22 (33.84%), and *Micrococcus Lyle* 3 (4.62%), however the percentage of Gram-negative bacteria: *E. coli* 11 (16.9%), *Serrati Marcescens* 4 (6.2%) (table 2, figure 5).

**Table (1): distribution of groups regarding the results of bacterial semen cultures (n =186 Cases):**

| Semen samples | No of Cases | Percentage (%) |
|---|---|---|
| **Infected semen sample** | 65 | 34.94% |
| **Non-infected semen sample** | 121 | 65.06% |

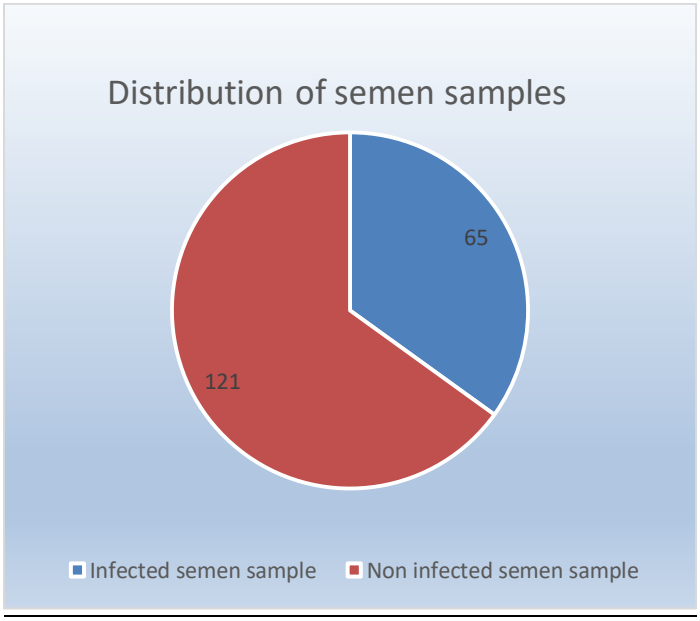


**Fig (4): distribution of groups regarding the results of bacterial semen cultures**

**Table (2): distribution of bacterial strains isolated from semen cultures (n =65 Cases).**

| Bacterial strains | Pathogens | No of Cases | Percentage (%) |
|---|---|---|---|
| **Gram-Positive** | *Enterococcus Fecails* | 25 | 38.46% |
| | *Staphylococcus-Haemolyticyus* | 22 | 33.84% |
| | *Micrococcus lylae* | 3 | 04.6% |
| **Gram-Negative** | *E. coli* | 11 | 16. 9% |
| | *Serratia Marcescens* | 4 | 06.20% |

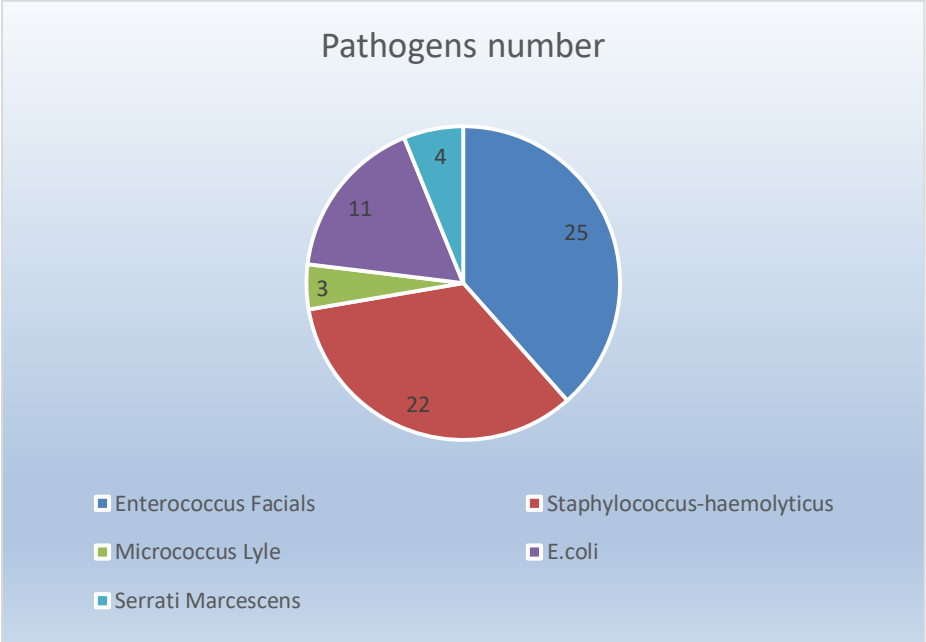


**Fig (5): distribution of bacterial strains isolated from semen cultures**

The results showed the negative impact of bacterial infection on human sperm parameters. The indices of sperm count, sperm progressive %, motility, & vitality, were higher in the non-infected group compared to the infected group but this variation was not statistically significant. The indices of sperm abnormality, sperm head, Midpiece, and tail defects, were lower in the non-infected group than in the infected group with insignificant statistical variance (table 3, figure 6).

In the Comparison of sperm functions between non-infected sub-fertile men and the infected group, the results illustrated present the negative influence of bacterial infection on human sperm function compared to non-infected sub-fertile men. high Reactive oxygen species (ROS) with low HOST and Acrosin Activity (A.A) values in infected sub-fertile men as compared to non-infected sub-fertile men but with no significance (table 4, figure 7).

**Table (3): Comparative analysis of sperm factors among the Infected & non-infected group (n =186 Cases):**

| Sperm parameters | Infected group n = 65 | Non-infected group n=121 | P-Value |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | |
| **Sperm Count/million** | 29.20 ± 33.2 | 36.7 ± 36.5 | 0.165 |
| **Sperm Motility (%)** | 28.80 ± 19.1 | 33.2 ± 17.9 | 0.119 |
| **Progressive motility (%)** | 3.19 ± 3.8 | 3.87 ±3.19 | 0.497 |
| **Sperm Vitality (%)** | 57.80 ± 20.3 | 62.3 ± 17.7 | 0.135 |
| **Sperm Abnormality (%)** | 92.31 ± 7.04 | 90.84 ± 8.09 | 0.198 |
| **Head defects (%)** | 49.97 ± 7.97 | 49.8 ± 7.79 | 0.888 |
| **Midpiece defects (%)** | 18.83 ± 9.72 | 18.18 ± 9.31 | 0.657 |
| **Tail defects (%)** | 23.51 ± 7.78 | 22.99 ± 7.98 | 0.669 |

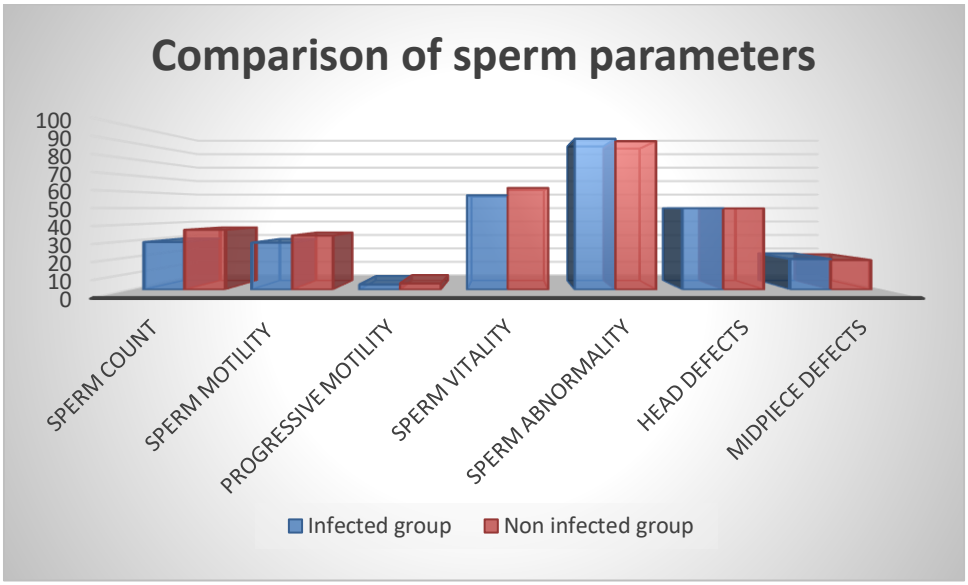


**Fig (6): Comparative analysis of sperm factors between Infected and Non-infected groups.**

**Table (4): Comparison of sperm functions between Non-infected and Infected group (n =186 Cases):**

| Sperm function test | Infected group n = 65 | Non-infected group n=121 | P-Value |
|---|---|---|---|
| | Mean ±SD | Mean ±SD | |
| **Hypo-osmotic swelling test (HOST)** | 40.7±17.8 | 42.7±18.1 | 0.497 |
| **Reactive oxygen species (ROS)** | 1.333±0.502 | 1.317±0.541 | 0.856 |
| **Acrosin Activity (A.A)** | 18.91±9.58 | 21.1±10.9 | 0.188 |

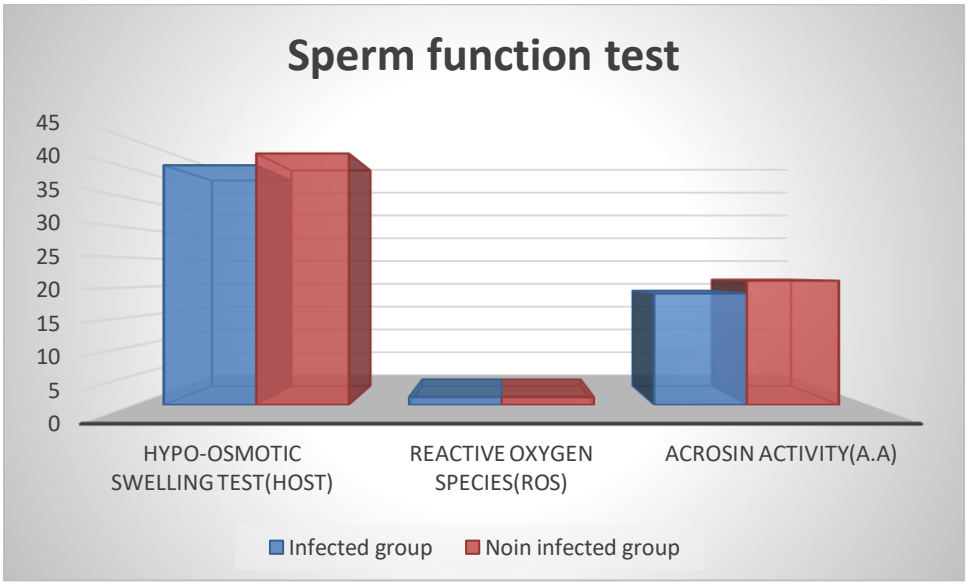


**Fig (7): Comparative analysis of sperm functions among Infected and Non-infected group**

## Discussion:

Infertility impacts ten to fifteen percent of couples within the reproductive age demographic. Roughly fifteen percent of infertility caused by male factors is attributed to infectious causes, including protozoa, viruses, fungus, & bacteria (23-25). The infiltration of germs into the male reproductive system correlates with diminished sperm functionality, resulting in infertility. A recent investigation indicated that sixty percent of participants using assisted reproductive technology (ART) had infection or inflammation. Bacteriospermia was directly associated with fifteen percent of infertility in men, presenting a significant issue in andrology (26-29). The presence of pathogenic organisms in semen samples adversely impacts sperm parameters, hence negatively influencing artificial reproduction methods. The quality of both sperm & oocytes is crucial for successful fertilization. Poor quality of sperm and/or oocytes diminishes the rate of fertilization. The presence of bacteria in semen samples is significantly associated with reduced sperm motility & recurrent pregnancy losses (30, 31). Bacteriospermia can disrupt normal fertility through genital tract obstruction caused by fibrosis and inflammation, the formation of anti-sperm antibodies due to the breach of the blood-testes barrier, the generation of reactive oxygen species resulting in an elevated DNA fragmentation index, altered sperm morphology, impaired acrosome reaction, reduced sperm motility, & reduced spermatogenesis (32). DNA damage in sperm is predominantly induced by reactive oxygen species during its transit through the male reproductive system. The prevalence of asymptomatic genitourinary tract infections prompts consideration of the necessity of treatment for the affected patients. Increasing data suggests a correlation between diminished semen quality & asymptomatic bacteriospermia (33, 34). The presence of significant fragmented DNA in infertile people has established sperm DNA integrity as a predictor of male fertility. The condensation of sperm DNA is a crucial element in male fertility, and the integrity of sperm DNA seems to affect early embryonic development (35, 36).

The primary objective of the research was to assess the effects of microbial infection on human sperm variables and functions to predict the fertilization potential of human spermatozoa. This study included only 186 couples among 200 couples who fulfilled the inclusion criteria. They have been separated into 2 groups: The infected group: males partner with infected semen, n = 65; the infected group: males partner with non-infected semen, n = 121. Regarding the percentage of bacterial cultures, the current study showed that there were 65 (34.94%) infected semen samples and 121 (65.06%) non-infected semen samples.

The current study, in agreement with Zeyad et al. (2018), aimed to investigate the impact of bacteriospermia on nuclear protamine levels, human sperm variables, outcomes of ICSI treatment, and DNA integrity. Microbiological analysis of semen specimens revealed that twenty-nine specimens (34.52%) had been contaminated, and 55 (65.48%) samples were non-infected (37). The research conducted by Shash et al. (2023) aimed to assess the influence of bacteriospermia on semen variables & sperm DNA fragmentation. They indicated that sixty-eight participants have been involved in thirty-four specimens of semen exhibiting bacteriospermia & thirty-four semen samples devoid of bacteriospermia (38). Likewise, Abbas et al. (2019) aimed to examine the impact of bacterial infections on male infertility in the Al-Anbar area of western Iraq. It was stated that eighty semen specimens had been obtained, with bacteriospermia detected in 42 (52.5%) of the samples (39).

Additionally, our results are corroborated by Eini et al. (2021), who aimed to examine the frequency of bacterial infections in sub-fertile males and their impact on semen quality. Sixty cases (34.88%) exhibited a positive culture for several kinds of harmful bacteria (40). According to the percentage of positive bacteria, our results revealed that (38.46%) were *Enterococcus facials*, (33.84%) were *Staphylococcus haemolyticus*, (4.6%) were *Micrococcus lyle*, and regarding the percentage of gram-negative bacteria, (6.2%) were *Serrati marcescens*, and (16.9%) were *E. coli*. In the microbiological assessment, the research by Shash et al. (2023) indicated that *S. aureus* was the predominant organism, identified from twenty-three (67.6 percent) samples, followed by *E. coli* in five (14.7 percent) samples, Klebsiella spp. in four (11.8 percent) samples, and Enterococcus in two (5.9 percent) samples. (37). Also, according to isolated and identified bacteria, the study of Zeyad et al. (2018) revealed that 8 (27.5%) were *Staphylococcus aureus*, 5 (17.2) *Staphylococcus epidermidis*, 4 (13.7%) *Staphylococcus haemolyticus*, 6 (20.6%) *Escherichia coli*, 4 (13.7%) *Enterococcus facials*, and 2 (6.89%) Streptococcus agalactiae. (36). Furthermore, regarding microbiological evaluation, the research conducted by Abbas et al. (2019) revealed that *Escherichia coli* (13.7 percent) was the predominant isolated organism, succeeded by Coagulase-negative Staphylococcus (ten percent), *Klebsiella pneumonia* (11.2 percent), *Streptococcus pyogenes* (6.2 percent), *Staphylococcus aureus* (7.5 percent), and *Pseudomonas aeruginosa* (3.7 percent) (39).

Our study showed that there was a negative impact of infection with bacteria on human sperm parameters. The indices of sperm count, sperm progressive %, motility, vitality, and sperm normality were higher in the non-infected group than in the infected group, but this variation was not statistically significant. The indices of sperm abnormality, sperm head, midpiece, and tail defects were lower in the non-infected group than in the infected group, but with no significant statistical differences. According to the negative impact of infection with bacteria on sperm quality, Zeyad et al. (2018) revealed that the mean levels of sperm concentration were 24.74 ± 15.86, motility was 25.74 ± 19.11, and progressive motility was 05.16 ± 07.31 in bacteriospermic patients. In non-bacteriospermic patients, the mean levels of sperm concentration were 76.08 ± 50.96, motility 50.52 ± 18.53, and progressive motility 22.49 ± 12.14. The

mean level of sperm, motility, & progressive motility was significantly diminished (p less than 001) in bacteriospermic cases relative to non-bacteriostatic patients. Other metrics exhibited no significant changes among the two groups (37). The current research aligns with Shrestha et al. (2023), who investigated the rate of infection in the semen of infertile men & the correlation between seminal bacteria & semen characteristics pertinent to reproductive potential. Their findings indicated that total motility, sperm concentration, morphology, & vitality of samples are often diminished in males with bacteriospermia compared to those without; however, the link was statistically inconsequential, with p-values beyond 0.05 (41). The research conducted by Berjis et al. (2018) was to investigate the impact of bacterial infection on semen characteristics, involving count, motility, & normal morphology, in infertile male cases. The investigation comprised 150 infertile guys with abnormal semen parameters (study group) & 150 healthy fertile males (control group). The average sperm count in each group was significantly less than that of the control group. In the group of infertile men without bacterial infection, sperm volume was diminished, but not to a statistically significant degree (42). The present investigation contradicts Eini et al. (2021), who demonstrated that sperm concentration & motility were significantly diminished in infected samples compared to non-infected ones (40).

The present study revealed that indices of sperm function tests, including HOS-test, ROS-test, acrosin activity, and DNA fragmentation index, were higher in the non-infected group than the infected group, but this variation wasn't statistically significant. In contrast, our findings disagreed with Zeyad et al. (2018), who exhibited that the mean DNA fragmentation in the infected group was 18.84 ± 09.47 while in the non-infected group, it was 14.52 ± 07.58. They revealed that the mean DNA fragmentation in the infected group was non-significantly greater than in non-infected patients (37). Also, our results disagreed with Eini et al. (2021), who established that sperm DNA fragmentation was significantly greater in infected samples compared to non-infected ones (40).

## Conclusion:

Our results revealed that there was a negative impact of infection with bacteria on human sperm parameters. The indices of sperm motility, count, sperm progressive%, vitality, and sperm normality were higher in the non-infected group than in the infected group, but this variance was not statistically significant. The indices of sperm abnormalities were lower in the non-infected group than in the infected group, with statistically insignificant variations observed. Based on our present findings, we conclude that male partner cases should have a semen bacterial analysis and thereafter address any identified bacteriospermia before ICSI treatment. Further investigation into this matter is essential.

## List of abbreviations:

**A.A**: Acrosin activity
**ART:** Assisted Reproduction Technology.
**DNA:** Deoxyribonucleic Acid
**HPF:** High Power Field
**HOST:** Hypo-Osmotic Swelling Test
**MDA**: Malondialdehyde
**ROS:** Reactive Oxygen Species.
**SPSS:** Statistical Package for the Social Sciences
**WHO:** The World Health Organization

## Declaration:

### Ethical approval:

The ethical research committee of the International Islamic Center for Population Research & Studies at Al-Azhar University in Cairo, Egypt, confirmed the present work. This has been conducted in accordance with the ethical requirements of the 1964 Helsinki Declaration and its subsequent comparable ethical standards or revisions, along with the ethical standards of the national and/or institutional research committee. All couples completed the informed consent forms for this research.

**Consent for publication:** All authors read & permitted the final manuscript. All participants in our investigation provide their written permission before the data is maintained and processed in a private, anonymous manner.

**Availability of data and materials:** All data & materials produced or examined during this investigation are contained within this published paper and its additional information files.

**Competing interests**: The authors declare that they haven't conflicting interests.

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