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The Role of Bacterial Infection in Sperm Parameters and DNA Fragmentation in Infertile Men

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ABSTRACT

Background and Aim: Infertility is an increasing problem worldwide, and earlier bacterial infections are unrecognized but potentially modifiable risk factors. Their impact on sperm health must be understood to create targeted therapies. This study aimed to investigate the role of bacterial infection in male infertility.

Materials and Methods: Seminal fluid samples were obtained from 64 male individuals who attended the Infertility and IVF Treatment Center in Al-Kafeel Hospital, Karbala. All samples were assessed for the sperm parameters, its DNA fragmentation, and bacterial infection.

Results: Among 64 infertile men, 31 (48%) had positive bacterial culture of various species. The highest percentage was among Gram-positive bacteria (51.6%). Sperm progressive motility and morphology showed a significant association with bacterial infection ($P<0.05$). Furthermore, a substantial risk of sperm DNA fragmentation was seen in contaminated semen samples. According to the analysis of the effect of each bacterial species on sperm characteristics, the results showed that *Enterobacter spp*. had the greatest effect on sperm immobilization, while coagulase-negative *Staphylococcus* (CoNS) showed a high rate of abnormal morphology and DNA fragmentation, indicating their severe impact on sperm morphology and DNA.

Conclusion: These findings underscore the need for early detection and targeted antimicrobial strategies to reduce the adverse effects of infections on male reproductive health and fertility. Further investigation is necessary to explore the potential treatment options and improve the reproductive outcomes in affected individuals.

Keywords: Bacterial Infection, DNA Fragmentation, Male Infertility, Sperm Quality

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1. Introduction

Male infertility has increased in recent years, primarily due to sperm quality reductions. Between 2013 and 2021, the ratio of infertile males to infertile females grew from 3:7 to 5:5 **(1)**. In 50% of couples, the male factor is the primary cause of infertility, which affects spermatogenesis, including congenital and acquired urogenital abnormalities, male accessory gland infections, elevated scrotal temperature, endocrine disorders, genetic abnormalities, and immunological issues **(2)**. Among these factors, infections and inflammatory conditions remain the most important reasons for infertility **(3)**.

Microorganisms can impact the function of spermatozoa in several ways including (a) Direct interaction with sperm cells, motile sperm agglutination, reduced ability of the acrosome reaction, and changes in cell morphology with the help of pili **(4)**. (b) Initiate a localized inflammatory response that raises reactive oxygen species (ROS) **(5)**. (c) Sperm autoantibody induction **(6)**. (d) Cytotoxic factor production. (e) Long-term antibiotic therapy of an infection that may result in sperm abnormalities **(7)**.

Escherichia (E.) coli, Klebsiella spp., *Staphylococcus (S.) aureus, Streptococci*, *Chlamydia (C.) trachomatis*, *Mycoplasma (M.) hominis,* and *Enterococcus* spp. are the most common bacteria isolated from semen samples **(3)**.

Sperm parameters and DNA integrity are severely impacted by the presence of these bacterial infections **(7)**. The conventional approach to diagnose male infertility involves examining the sperm motility, concentration, and morphology under the microscope. These tests are necessary to give the fundamental details about the quality of the sperm **(8)**. Tests for sperm DNA fragmentation (SDF) can distinguish between those who are fertile and infertile. Elevated SDF levels are positively connected with decreased IVF (*in vitro* fertilization) fertilization rates, decreased implantation rates, Offspring birth abnormalities, and premature births. It might therefore be a more impartial indicator of sperm function **(9, 10)**. It is currently unknown which biological process the bacteria use to alter chromatin and sperm nuclear protein. Histone H3 methylation and hyperacetylated H4 both at lysine 79 prematurely arise as a result of bacterial infections. In mammals, decreased fertility is correlated with lower levels of histone H4 hyperacetylation **(7)**.

This study aimed to investigate the impact of bacterial infections on sperm characteristics and its DNA integrity in infertile men. The specific objectives are to isolate and identify bacterial pathogens in semen samples from infertile men and evaluate the effects of bacterial infections on sperm motility, morphology, and concentration, and assess the rate of sperm DNA fragmentation in infected and non-infected samples.

2. Materials and Methods

2.1 Sample Size and Semen Samples Collection

Semen samples (n=64) from men who were admitted to the Infertility and IVF Treatment Center in AL-Kafeel Hospital, Karbala, during the period from 1 October to 31 December 2024. Male participants with medical or surgical conditions such as fever, varicocele, or hydrocele that could affect semen parameters were excluded from the study. The approval from the Institutional Ethical Committee was acquired from Al-Kafeel Hospital (3785, 2024-9-24). Following the protocol outlined in the World Health Organization (WHO) Manual **(11)**, the samples were collected in sterile containers, allowed to liquefy, and then examined as a wet mount under microscope for evaluation of sperm motility and count. The morphology and chromatin integrity of the sperm were also examined.

2.2 Sperm Morphology

The slides were stained with Papanicolaou dye in order to identify the sperm morphology and sperm deformity index (SDI). Under ×100 magnification, two hundred sperm were examined and classified as normal or abnormal. Next, the SDI and abnormal sperm percentages were determined **(11)**.

2.3 Sperm Chromatin

The feathering method was used to prepare semen slides in order to assess the chromatin integrity of the sperm. After fixation in 3% glutaraldehyde for 30 min, they were stained with acidic aniline blue for 5 min. The stained slides were observed under ×100 magnification. Dark blue-stained sperm were considered aberrant or to have broken DNA, but sperm with unstained or faintly stained nuclei were considered normal. The DNA fragmentation index (DFI), or the quantity of fragmented sperm per 100 sperm, was calculated as a percentage of 200 sperm that were counted **(12)**.

2.4 Microbial Culture and Evaluation

In order to differentiate between urinary tract infection and seminal tract infection, the patients were trained before seminal collection. The semen samples were diluted in sterile saline (1:10) and 1 ml of each sample was centrifuged at 1500 rpm for 15

min at room temperature. The sediment was suspended in 100 µL of sterile saline solution after the supernatant was removed. This procedure increases the cultural sensitivity by concentrating bacteria in the cell pellet and eliminating the seminal plasma. For aerobic bacteria, the cell pellet was placed on blood agar and McConkey agar and for fastidious bacteria it was spread on chocolate agar.

All media were incubated at 37°C for 24 and 48 hr. The colonies development was initially identified according to the formal characteristics of colonies, including the size, color, piles, and height of the colony. The isolates of the bacteria were examined in several biochemical and physiological tests to determine their genus. The identification was also carried out using the VITEK-2 equipment (bioMérieux, France) following the manufacturer's instructions.

The subsequent criteria were used to score the bacterial load: reduced ($<10^3$ CFU/mL), mild ($>/=10^3$ CFU/mL), moderate (10^3–10^4 CFU/mL) and severe ($>10^4$ CFU/mL) with scores 0, 1, 2, and 3, respectively. The infective value for the examined semen samples was calculated by adding the scores (bacterial load plus etiological agent); if the result was more than 3, the sample was considered infected; if it was equal to or less than 3, it was considered non-infected **(13)**.

2.4 Statistical Analysis

The statistical analysis was carried out utilizing IBM SPSS Statistics version 23. The analytical results were subsequently presented adopting descriptive statistics. Data were presented as mean±SD. A probability threshold of $P<0.05$ was employed to assess the statistical significance of the experimental results. Furthermore, the Shapiro-Wilk test was utilized to assess data normality, while the Levene test was performed to evaluate variance homogeneity. Chi-square and Pearson's correlation analyses were carried out to investigate the association between categorical and numerical variables, respectively. The Mann-Whitney Test and Independent T-Test were employed to ascertain statistical differences between two distinct sets of data. The analysis of variance (ANOVA) was utilized to do multiple comparisons among the groups. The Duncan post-hoc test was employed at significance level of $P<0.05$ for multiple groups comparison.

3. Results

3.1 Bacterial Analysis of Semen Samples

The bacteriological analysis of semen samples demonstrated 33 samples (52%) as sterile and 31 samples (48%) with bacterial growth, indicating a substantial prevalence of semen infection among the studied population (Figure 1). Among the identified bacterial isolates, Gram-positive bacteria were the most prevalent, accounting for 51.6% of the infections, while Gram-negative bacteria constituted 48.4% (Figure 2).

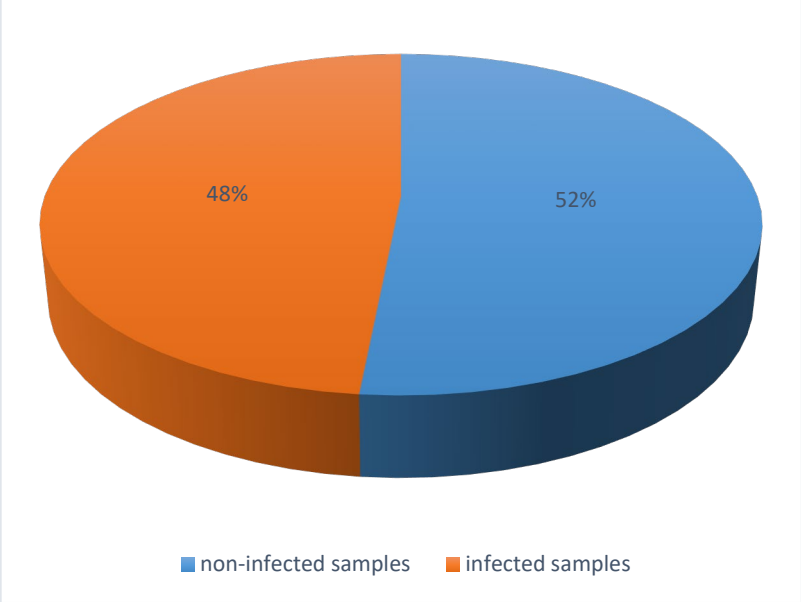

Figure 1. The percentages of infected and non-infected semen samples.

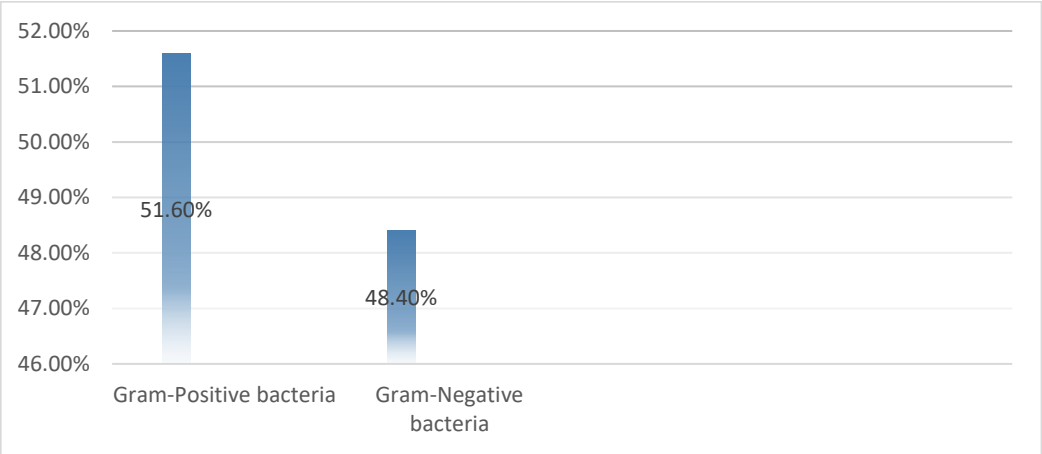

Figure 2. The percentages of Gram-positive and Gram-negative bacteria in semen samples.

Among positive cultures, *Staphylococcus aureus* was the most prevalent bacterium with 25.81%. *Klebsiella spp.* followed closely at 22.58%.CoNS represented 19.35%. *E. coli* was found in 16.13% of cases (Table 1).

Table 1. Distribution of bacterial growth according to the species.

Bacterial species	Percentage (%)	P-value
E. coli	16.13	
Enterobacter spp.	6.45	
Enterococcus spp.	9.68	
Klebsiella spp.	22.58	0.393
Staphylococcus aureus	25.81	
CoNS	19.35	
Total	100.00	

3.2 The Effect of Age and Smoking on Bacterial Infection

Smoking showed no significant impact on bacterial culture results (P>0.05), though non-smokers showed slightly higher rate of positive cultures **[21]**, compared to smokers **[10]** (Table 2).

The similar finding was obtained with age. The age had no significant effect on bacterial culture, although younger individuals (<35) had a slightly higher percentage of positive cultures (58.06%), than older individuals (≥35) (41.94%).

Table 2. Distribution of bacterial infections according to age and smoking.

Parameters	Level	Bacteria culture		Total	P-value
		Negative	Positive		
Smoking	**Non-Smoker**	19	21	40	0.401
		57.58%	67.74%	62.50%	
	Smoker	14	10	24	
		42.42%	32.26%	37.50%	
Age Group	**<35**	14	18	32	0.211
		42.42%	58.06%	50.00%	
	≥35	19	13	32	
		57.58%	41.94%	50.00%	

3.3 The Effect of Bacterial Infection on Semen Parameters

Table 3 presents the effects of bacterial infection on sperm parameters in infected samples compared to non-infected. Sperm Progressive, Motility, and DNA fragmentation had the lowest P-value (<0.01), indicating that bacterial infection is significantly associated with decreased sperm quality and DNA integrity. On the other hand, the infections related to uncommon sperm morphology (P<0.05) suggest potential damage to the sperm structure.

Table 3. Sperm parameters in positive and negative bacterial cultures.

Parameters	Bacteria culture						P-value
	Negative			Positive			
	Mean	Std. Deviation	Std. Error	Mean	Std. Deviation	Std. Error	
Concentration	21.45	8.12	1.41	23.42	11.63	2.09	0.81
Progressive	37.879	5.16	0.90	33.32	6.82	1.23	0.005
Non- progressive	32.79	6.34	1.10	31.52	5.65	1.02	0.469
Immobile	28.58	8.57	1.49	35.16	6.52	1.17	0.002
Normal morphology	18.82	9.48	1.65	13.48	5.70	1.02	0.029
DNA- fragmentaion	0.30	0.10	0.02	0.38	0.12	0.02	0.006

3.4 Comparison of Semen Parameters Based on Bacterial Species

Lower sperm concentrations were obtained in *E. coli, Enterobacter spp., Klebsiella spp., S. aureus*, and CoNS, suggesting that these bacterial infections may negatively impact sperm production. The means of sperm concentration were almost similar, with no significant difference between them. On the other hand, *Enterococcus* had the lowest effect on this parameter (Table 4).

Tables 5 and 6 illustrate no clear evidence of the effect of bacterial species on progressive and non-progressive motility. Bacteria may reduce the overall sperm motility, but the type of bacteria does not seem to cause significant variation in progressive motility.

Table 4. Sperm concentration according to the bacterial species.

Bacterial species	Mean	Std. Deviation	Std. Error	P-value	Post hoc*
E. coli	20.00	3.54	1.58	0.044	a
Enterobacter spp.	17.50	3.54	2.50		a
Enterococcus spp.	41.67	16.07	9.28		b
Klebsiella spp.	26.29	15.11	5.71		a
Staphylococcus aureus	21.50	9.67	3.42		a
CoNS	18.33	4.08	1.67		a

*similar letters (no significant), different letters (significant)

Table 5. Sperm progressive according to the bacterial species.

Bacterial species	Mean	Std. Deviation	Std. Error	P-value	Post hoc*
E. coli	32.00	4.47	2.00	0.871	a
Enterobacter spp.	30.00	0.00	0.00		a
Enterococcus spp.	37.67	2.52	1.45		a
Klebsiella spp.	32.86	4.88	1.84		a
Staphylococcus aureus	33.13	10.67	3.77		a
CoNS	34.17	7.36	3.01		a

*similar letters (no significant)

Table 6. Sperm non-progressive according to the bacterial species.

Bacterial species	Mean	Std. Deviation	Std. Error	P-value	Post hoc*
E. coli	32.00	4.47	2.00	0.752	a
Enterobacter spp.	30.00	0.00	0.00		a
Enterococcus spp.	35.67	4.04	2.33		a
Klebsiella spp.	31.43	6.27	2.37		a
Staphylococcus aureus	31.88	7.99	2.83		a
CoNS	29.17	3.76	1.54		a

*similar letters (no significant)

Bacterial species showed significantly different impact on sperm immobility (*P*=0.04235) (Table 7). *E. coli, Klebsiella spp., Staphylococcus aureus*, and CoNS species showed similar effects (all means between 35-37% immobility) with no significantly difference from each other. *Enterobacter spp*. showed the highest sperm immobility (40%), indicating that it may have a stronger negative effect on sperm motility.

Among the bacterial species analyzed, CoNS exhibited the most severe impact on sperm morphology, with the lowest recorded normal morphology percentage (7.83%) (Table 8). This finding suggests that CoNS infections may significantly compromise sperm structural integrity, potentially affecting the fertilization ability. *Klebsiella spp*. and *Staphylococcus aureus* demonstrated moderate effects on sperm morphology, indicating a partial impairment in sperm structure.

The analysis of SDF levels revealed that *E. coli* and CoNS species applied the highest levels of DNA fragmentation (0.45–0.47) (Table 9) with significant sperm DNA damage. In contrast, *Enterobacter spp*., *Klebsiella spp*., and *Staphylococcus aureus* demonstrated intermediate effects on SDF, indicating a moderate impact on sperm genetic stability.

Table 7. Sperm immobility according to the bacterial species.

Bacterial species	Mean	Std. Deviation	Std. Error	P-value	Post hoc*
E. coli	36.00	5.48	2.45	0.042	ab
Enterobacter spp.	40.00	0.00	0.00		b
Enterococcus spp.	26.67	5.77	3.33		a
Klebsiella spp.	35.71	4.50	1.70		ab
Staphylococcus aureus	35.00	7.56	2.67		ab
CoNS	36.67	7.53	3.07		ab

*similar letters (no significant), different letters (significant)

Table 8. Sperm morphology according to the bacterial species.

Bacterial species	Mean	Std. Deviation	Std. Error	P. value	Post hoc*
E. coli	16.60	4.22	1.89	0.021	b
Enterobacter spp.	17.50	3.54	2.50		b
Enterococcus spp.	18.33	2.89	1.67		b
Klebsiella spp.	15.00	7.07	2.67		ab
Staphylococcus aureus	11.63	4.75	1.68		ab
CoNS	7.83	2.48	1.01		a

*similar letters (no significant), different letters (significant)

Table 9. The SDF according to the bacterial species.

Bacterial species	Mean	Std. Deviation	Std. Error	P-value	Post hoc*
E. coli	0.47	0.12	0.05	0.033	b
Enterobacter spp.	0.33	0.03	0.02		ab
Enterococcus spp.	0.26	0.02	0.01		a
Klebsiella spp.	0.37	0.14	0.05		ab
Staphylococcus aureus	0.34	0.13	0.05		ab
CoNS	0.46	0.09	0.04		b

*similar letters (no significant), different letters (significant)

4. Discussion

The present study highlights the significant impact of bacterial infections on sperm quality and DNA integrity, reinforcing the growing evidence that microbial colonization of the male reproductive tract can contribute to infertility. Our findings align with previous research suggests that bacterial infection adversely affects the sperm motility, morphology, and DNA integrity, ultimately impairing male fertility outcomes (**9, 14- 16**).

Pathogenic bacterial cultures were detected in 48% of the semen samples, with the Gram-positive bacteria most prevalent, which indicates Gram-positive organisms among the most frequent contaminants and opportunistic pathogens in semen infections. These results were consistent with investigations of earlier studies by Eini et al **(9)** and Nasrallah et al **(17)** that found Gram-positive bacteria as the predominant pathogen associated with semen infections.

Nasrallah et al (**17**) reported that among detected species, *Staphylococcus aureus* was the predominant species with 46.2%, followed by urogenic Gram-negative pathogens (24.1%). This outcome is consistent with our observations. Staphylococcal species are commensal microorganisms with relative pathogenic significance in reproductive tract infections **(18)**. The primary way of bacterial invasion to reproductive organs could be direct ascending infection, or by hematogenous spread of the bacteria. After entry, Toll-like receptors (TLRs), a type of pathogen recognition receptor on the host cells, recognize the pathogen-associated molecular patterns (PAMPs) of Staphylococci and initiate inflammatory signaling cascades in testicular cells, epididymis, and other regions of the male reproductive system **(19)**. This immune response significantly contributes to the damage of tissue, defects in sperm function, and hence the concept of infertility **(19, 20)**

However, the present study contrasts with 21. Bhatt et al **(21)**, where Gram-negative bacteria were the main pathogens (62.9%) of all infections and *E. coli* was the most frequently isolated bacterium (41.9%). This discrepancy in bacterial diversity could be for the study population differences, different sampling procedures, and diagnostic differences for bacterial identification.

Analysis of the other factors effect on seminal bacterial infections, such as smoking and age, brings to a standstill that smoking does not have a significant association with the prevalence of seminal infections. This is consistent with the results of De Bantel et al **(22)** and Keskin et al **(23)** studies. However, some previous studies **(24, 25)** implicate smoking as relatively more prone to infection, particularly through immune suppression and increased oxidative stress that undermines the host immunity. The lack of a significant association in the current study could be explained by the low number of smokers in this study samples that could have reduced the statistical power to find a significant effect.

In contrast, age appeared to be a more influential factor, as younger participants (<35 years) exhibited a higher rate of bacterial positivity compared to older individuals (≥35 years). This trend may suggest that younger men are more exposed to environmental or behavioral risk factors, including increased sexual activity, hygiene practices, and a higher likelihood of prior infections, all of which could contribute to increase the bacterial colonization **(26)**.

Studying the impact of bacterial infections on the parameters of semen highlighted their adverse impact on sperm morphology, and motility, which are key factors for fertility. These findings indicate that bacterial infections contribute to male infertility by impairing the sperm functionality. There is a great debate over the effect of the bacteria that contaminate and colonize the male urogenital tract to decrease the sperm parameters **(9)**. According to Fraczek and Kurpisz **(27)**, bacterial infections decreased human spermatozoa and the ability of sperm cells for fertilization due to immobility and

shape alteration of spermatozoa. Nevertheless, the massive influx of activated leukocytes to the inflamed site can be associated with decreased sperm fertilizing capability owing to biological oxidative, apoptotic, and inflammatory events around spermatozoa **(28)**.

In the present study, a significant increase in Sperm DNA Fragmentation (SDF) was shown in semen samples infected by bacteria, which may explain the influence of microbial infections on sperm DNA integrity. Importantly, it was shown before that clear associations were observed between SDF levels and the presence of microbial agents in semen, suggesting that bacterial presence may exacerbate oxidative stress and inflammation, thus ultimately leading to DNA damage **(9, 29)**.

The integrity of sperm DNA is becoming one of the most crucial aspects of male fertility. Numerous studies have shown that infertile people have a higher rate of DNA fragmentation than fertile controls **(30, 31)**. Reactive oxygen species (ROS), which are significantly elevated in the presence of bacteria, appear to be a key pathogenic mechanism **(32)**. As leukocytes overproduce ROS, which can act directly on sperm DNA and degrade sperm functioning, the impact of oxidative damage and increased exposure to leukocytes is remarkable in the presence of infection **(1)**.

Aitken and De Iuliis **(33)** illustrated that sperm DNA fragmentation may cause infertility, birth defects in offspring, and miscarriage. Sperm DNA damage can result from two different kinds of factors, including intrinsic and extrinsic variables. Intrinsic variables consist of oxidative stress, apoptosis, and failure in histone protamine replacement. They are present in ejaculates **(34)**. If chromatin packing is not finished during sperm maturation, sperm DNA is exposed to damage **(8)**. Extrinsic variables include handling conditions, storage temperatures, post-testicular oxidative stress, infections, ejaculation timing, and medication reactions **(7)**.

However, the impact of bacterial infections on individual semen parameters varies depending on the specific bacterial species involved. In particular, sperm concentration appears to be negatively affected with lower sperm counts observed in samples infected with *E. coli*, *Enterobacter spp*., *Klebsiella spp*., *Staphylococcus aureus*, and CoNS. These findings suggest that bacterial infections may disrupt spermatogenesis or impair sperm viability, potentially through inflammatory responses or oxidative stress. Similarly, Eini et al (9) reported that infected infertile samples exhibited significantly lower sperm concentration, motility, and morphology compared to non-infected samples, reinforcing the role of microbial infections in deteriorating semen quality. *E. coli* strains are recognized for their capacity to immobilize and damage spermatozoa morphology with direct contact via attachment organelles like pili or type-1 fimbriae, and mannose receptor-dependent interactions **(35)**.

Bacterial infections appear to reduce the overall motility, though the degree of impact does not significantly vary among bacterial species. However, *Enterobacter spp*. demonstrated the highest rate of sperm immobility (40%), suggesting a more pronounced inhibitory effect on sperm movement. *Enterococcus faecalis* was reported by Ho et al **(32)** as the most prevalent organism in semen (22.0% of samples), causing a significant increase in seminal ROS.

Different bacterial species, including *Streptococci pyogenes*, *Enterococci*, *E. coli*, and *staphylococci*, were isolated from 34.4% of semen samples by Khalili and Sharifi-Yazdi **(36)**, and sperm motility and morphology were negatively impacted by these bacteria. Normal sperm morphology significantly reduced by CoNS species, leading to more sperm abnormalities. *Klebsiella* spp. and *Staphylococcus aureus* showed moderate effects, indicating some degree of sperm morphology impairment. Fraczek et al **(37)** found that sperm ability to fertilize was decreased when they were incubated with bacteria and/or leukocytes, which had an adverse effect on sperm motility and lipid bilayers in their membranes.

E. coli and CoNS bacteria are associated with the highest sperm DNA fragmentation. These bacteria may produce toxic metabolites like ROS, or induce inflammation, contributing to the sperm DNA damage. *Klebsiella* spp., *Staphylococcus aureus*, and *Enterobacter* spp. showed moderate effects, suggesting a potential but not extreme risk to DNA integrity. Compared to other infected groups, sperm DNA fragmentation was noticeably higher in those with *S. aureus*, *K. pneumoniae*, and multi-bacterial infections **(9)**.

5. Conclusion

This study demonstrated a strong association between bacterial infections and impaired semen quality, with Gram-positive bacteria being the most prevalent pathogens. Notably, CoNS and *E. coli* were linked to severe sperm morphology abnormalities and DNA fragmentation. These findings emphasize the importance of early diagnosis and targeted antimicrobial interventions to minimize the bacterial-induced damage and improve male fertility outcomes. Further research is needed to explore the effective treatment strategies and enhance the reproductive success in affected individuals.

6. Declarations

6.1 Acknowledgment

We would like to thank all the health workers in Al-Kafeel Hospital for their support and cooperation.

6.2 Ethical Considerations

The protocol for this Research was approved by the University of Kufa. The approval from the Institutional Ethical Committee was acquired from Al-Kafeel Hospital (Ref.No 3785, 2024-9-24).

6.3 Authors' Contributions

All authors contributed to the study's conception and design. Huda N. Al-Baroody performed the laboratory tests and drafted the manuscript. Ali Ibrahim Rahim was responsible for sample collection and assisted with laboratory procedures. Etab Abdul-Ameer Al-Ogla conducted the statistical analysis. Thulfiqar Ibrahim Rahim performed the final review of the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

6.4 Conflict of Interests

The authors have no conflicts of interest to declare.

6.5 Financial Support and Sponsorship

This research received no specific grant from any funding agency.

6.6 Using Artificial Intelligence Tools (AI Tools)

None.

References

1. Liu KS, Mao XD, Pan F, An RF. Effect and mechanisms of reproductive tract infection on oxidative stress parameters, sperm DNA fragmentation, and semen quality in infertile males. Reprod Biol Endocrinol. 2021;19:1-2. [DOI:10.1186/s12958-021-00781-6]
2. Agarwal A, Makker K, Sharma R. Clinical relevance of oxidative stress in male factor infertility: an update. Am J Reprod Immunol. 2008;59(1):2-11. [PMID] [DOI:10.1111/j.1600-0897.2007.00559.x]
3. Farsimadan M, Motamedifar M. Bacterial infection of the male reproductive system causing infertility. J Reprod Immunol. 2020;142: 103183. [DOI:10.1016/j.jri.2020.103183] [PMID]
4. Sukarjati S, Kusuma PS, Binawati DK. An extract of protein pili and filtrat of human spermatozoa: A chemical review. In Journal of Physics: Conference Series 2020. Vol. 1469, No. 1, pp. 012003. Bristol, England: IOP Publishing. [DOI:10.1088/1742-6596/1469/1/012003]
5. du Plessis SS, van Zijl Drive F. The effect of streptozotocin induced diabetes on sperm function: A closer look at AGEs, RAGEs, MAPKs and activation of the apoptotic pathway. The Possible Ameliorating Effects of Rooibos, Honeybush and Sutherlandia on Diabetes-Induced Reproductive Impairment in Adult Male Wistar Rats. 2020. pp. 182.
6. Schuppe HC, Pilatz A, Meinhardt A, Behre HM. Infections and inflammation of the seminal ducts and accessory sex glands. InAndrology: Male Reproductive Health and Dysfunction. 2023. pp. 353-71. Cham, Germany: Springer International Publishing. [DOI:10.1007/978-3-031-31574-9_26]
7. Zeyad A, Amor H, Hammadeh ME. The impact of bacterial infections on human spermatozoa. Int J Womens Health Reprod Sci. 2017;5(4):243-52. [DOI:10.15296/ijwhr.2017.43]
8. González-Marín C, Gosálvez J, Roy R. Types, causes, detection and repair of DNA fragmentation in animal and human sperm cells. Int J Mol Sci. 2012;13(11):14026-52. [DOI:10.3390/ijms131114026] [PMID] [PMCID]
9. Eini F, Kutenaei MA, Zareei F, Dastjerdi ZS, Shirzeyli MH, Salehi E. Effect of bacterial infection on sperm quality and DNA fragmentation in subfertile men with Leukocytospermia. BMC Mol Cell Biol. 2021;22: 1-10. [DOI:10.1186/s12860-021-00380-8] [PMID] [PMCID]
10. Lourenço ML, de Moura GA, Rocha YM, Rodrigues JP, Monteiro PB. Impact of sperm DNA fragmentation on the clinical outcome of assisted reproduction techniques: a systematic review of the last five years. JBRA Assist Reprod. 2023;27(2):282. [PMID] [PMCID] [DOI:10.5935/1518-0557.20220057]

11. World Health Organization. WHO laboratory manual for the examination and processing of human semen. InWho laboratory manual for the examination and processing of human semen. 2021.
12. Patil PS, Mudiraj NR, Mane VP. Aniline blue staining method for sperm DNA fragmentation index: A guide to sperm quality and infertility management-An observational study in males from South Maharashtra. Natl J Clin Anat. 2021; 10(3):126-30. [DOI:10.4103/NJCA.NJCA_31_21]
13. La Vignera S, Condorelli RA, Vicari E, Salmeri M, Morgia G, Favilla V, et al. Microbiological investigation in male infertility: a practical overview. J Med Microbiol. 2014;63(1):1-4. [DOI:10.1099/jmm.0.062968-0] [PMID]
14. Hassan EA, Sadeek YK, Abdelghany TM, Gadel-Rab AG. Influence of bacterial infection on human sperm. J Biosci Appl Res. 2024;10(6): 137-50. [DOI:10.21608/jbaar.2024.312999.1067]
15. Shash RY, Mohamed GA, Shebl SE, Shokr M, Soliman SA. The Impact of Bacteriospermia on Semen Parameters Among Infertile Egyptian Men: A Case-Control Study. Am J Men's Health. 2023;17(3):15579883231181861. [PMCID] [DOI:10.1177/15579883231181861] [PMID]
16. Pagliuca C, Cariati F, Bagnulo F, Scaglione E, Carotenuto C, Farina F, et al. Microbiological evaluation and sperm DNA fragmentation in semen samples of patients undergoing fertility investigation. Genes. 2021;12(5):654. [DOI:10.3390/genes12050654] [PMID] [PMCID]
17. Nasrallah YS, Anani M, Omar HH, Hashem AA. Microbiological profiles of semen culture in male infertility. Hum Androl. 2018;8(2):34-42. [DOI:10.21608/ha.2018.3207.1023]
18. Esmailkhani A, Akhi MT, Sadeghi J, Niknafs B, Bialvaei AZ, Farzadi L, et al. Assessing the prevalence of Staphylococcus aureus in infertile male patients in Tabriz, northwest Iran. Int J Reprod Biomed. 2018;16(7):469. [DOI:10.29252/ijrm.16.7.469] [PMID] [PMCID]
19. Dutta S, Sengupta P, Izuka E, Menuba I, Jegasothy R, Nwagha U. Staphylococcal infections and infertility: mechanisms and management. Mol Cell Biochem. 2020;474:57-72. [DOI:10.1007/s11010-020-03833-4] [PMID]
20. Li J, Li B, Song J, Liu H, Bi W, Dong G, et al. Characteristic and mechanism of immobilization effect of Staphylococcus aureus on human spermatozoa. Microb Pathog. 2018; 119:28-34. [DOI:10.1016/j.micpath.2018.03.049] [PMID]
21. Bhatt CP, Mishra S, Bhatt A, Lakhey M. Bacterial pathogens in semen culture and their antibiotic susceptibility pattern in vitro. Int J Biomed Res. 2015;6(11):909-14.
22. De Bantel A, Fleury-Feith J, Poirot C, Berthaut I, Garcin C, Landais P, et al. Simultaneous vitality and DNA-fragmentation measurement in spermatozoa of smokers and non-smokers. Cytom B - Clin Cytom. 2015;88(2):120-4. [DOI:10.1002/cytob.21185] [PMID]
23. Keskin MZ, Budak S, Gubari S, Durmaz K, Yoldas M, Celik O, et al. Do cigarette and alcohol affect semen analysis?. Arch Ital Urol Androl. 2016; 88(1):56-9. [DOI:10.4081/aiua.2016.1.56] [PMID]
24. Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization laboratory methods for the examination of human semen. Eur Urol. 2016;70(4):635-45. [DOI:10.1016/j.eururo.2016.04.010] [PMID]
25. Harlev A, Agarwal A, Gunes SO, Shetty A, du Plessis SS. Smoking and male infertility: an evidence-based review. World J Mens Health. 2015;33(3):143-60. [PMID] [PMCID] [DOI:10.5534/wjmh.2015.33.3.143]
26. Tesarik J. Lifestyle and Environmental Factors Affecting Male Fertility, Individual Predisposition, Prevention, and Intervention. Int J Mol Sci. 2025;26(6):2797. [DOI:10.3390/ijms26062797] [PMID] [PMCID]
27. Fraczek M, Kurpisz M. Mechanisms of the harmful effects of bacterial semen infection on ejaculated human spermatozoa: potential inflammatory markers in semen. Folia Histochem Cytobiol. 2015;53(3):201-17. [DOI:10.5603/fhc.a2015.0019] [PMID]
28. Ojo OA, Nwafor-Ezeh PI, Rotimi DE, Iyobhebhe M, Ogunlakin AD, Ojo AB. Apoptosis, inflammation, and oxidative stress in infertility: A mini review. Toxicol Rep. 2023;10:448-62. [DOI:10.1016/j.toxrep.2023.04.006] [PMID] [PMCID]
29. Haidl F, Haidl G, Oltermann I, Allam JP. Seminal parameters of chronic male genital inflammation are associated with disturbed sperm DNA integrity. Andrologia. 2015;47(4): 464-9. [DOI:10.1111/and.12408] [PMID]

30. Osman A, Alsomait H, Seshadri S, El-Toukhy T, Khalaf Y. The effect of sperm DNA fragmentation on live birth rate after IVF or ICSI: a systematic review and meta-analysis. Reprod Biomed Online. 2015;30(2):120-7. [DOI:10.1016/j.rbmo.2014.10.018] [PMID]

31. Simon L, Zini A, Dyachenko A, Ciampi A, Carrell DT. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. Asian J Androl. 2017;19(1): 80-90. [DOI:10.4103/1008-682X.182822] [PMID] [PMCID]

32. Ho CL, Vaughan-Constable DR, Ramsay J, Jayasena C, Tharakan T, Yap T, et al. The relationship between genitourinary microorganisms and oxidative stress, sperm DNA fragmentation and semen parameters in infertile men. Andrologia. 2022;54(2): e14322. [DOI:10.1111/and.14322] [PMID]

33. Aitken RJ, De Iuliis GN. On the possible origins of DNA damage in human spermatozoa. Mol Hum Reprod. 2009;16(1):3-13. [DOI:10.1093/molehr/gap059] [PMID]

34. Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, et al. Male infertility. Lancet. 2021;397(10271):319-33. [PMID] [DOI:10.1016/S0140-6736(20)32667-2]

35. Villegas J, Schulz M, Soto L, Sanchez R. Bacteria induce expression of apoptosis in human spermatozoa. Apoptosis. 2005;10:105-10. [DOI:10.1007/s10495-005-6065-8] [PMID]

36. Khalili MB, Sharifi-Yazdi MK. The Effect of Bacterial Infection on the Quality of Human's Spermatozoa. Iran J Public Health. 2001;30(3-4):119-22.

37. Fraczek M, Wiland E, Piasecka M, Boksa M, Gaczarzewicz D, Szumala-Kakol A, et al. Fertilizing potential of ejaculated human spermatozoa during in vitro semen bacterial infection. Fertil Steril. 2014;102(3):711-9. [DOI:10.1016/j.fertnstert.2014.06.002] [PMID]