



Assessment of DNA Fragmentation and Inhibin-B Hormone Levels in Infertile Men with Genital Tract Infections Caused by *Staphylococcus*



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Abstract

Background: No matter the test, DNA damage is caused by a break in nuclear material (DNA) and low semen quality. The likelihood of a normal pregnancy reduces dramatically when it reaches the limit. **Objective:** The purpose of the present study was to measure the sperm DNA fragmentation and inhibin B hormone levels in sick and healthy people to establish their link as well as to examine *Staphylococcus aureus* as a causative factor of genital tract infections and sperm DNA fragmentation. **Methodology:** This was designed as comparative cross-sectional study which was conducted in the Department of Forensic Science at College of Science, Al-Karah University for Science, Baghdad, Iraq, during the period from April 2023 to August 2025. An adult men aged between (17 and 51 years) were registered in this study. Microscopic examination of seminal fluid split the samples into three groups: low sperm count, normal sperm count, and control. We used a SCA scope to count fragmented and not fragmented sperm. Inhibin B levels were measured in all participants' blood. Seminal fluid samples cultivated on mannitol medium yielded *Staphylococcus aureus*. **Results:** A total number of 30 infected patients were recruited for this study of which 33.3% had low sperm counts, 33.3% cases were normal, and 33.3% healthy. We measured sperm nucleic acid fragmentation with SCASCOPE. Three groups had substantial differences in high and low nucleic acid fragmentation. Sperm fragmentation was highest in the third age group and lowest in the first. The three groups exhibited identical inhibin B levels. *Staphylococcus aureus* infected 54% and left 46% uninfected. Bacteria contaminated samples fragmented nucleic acids most. Bacteria lower inhibin B hormone levels while uninfected people raise them. **Conclusion:** Blood levels of inhibin B were strongly correlated with sperm nucleic acid fragmentation in this investigation. The study also showed that *Staphylococcus aureus*, a common source of genital tract infections, caused sperm deformity and DNA fragmentation. The need of early diagnosis utilizing inhibin B blood levels and treating infections with antibiotics to reduce damage and increase fertility to prevent sperm nucleic acid fragmentation in males is highlighted in this study. [Bangladesh Journal of Infectious Diseases, June 2026;13(1):79-86]

Keywords: *Staphylococcus aureus*; Inhibin B; DNA fragmented; SCA SCOPE

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Introduction

DNA damage results from a break in the nuclear material (single-stranded and double-stranded

nucleic acid), and its problems are related to poor semen quality¹, regardless of the tests used to detect such a pathological condition². When its level

exceeds the required limit, the probability of a normal pregnancy decreases significantly³. Approximately fifty million couples around the world are affected by infertility, with men being the primary cause in 50 % of the cases that have been documented. The most common cause is abnormally low levels of sperm production⁴. After being released from the seminal epithelium, sperm cells undergo a series of divisions throughout the process of sperm production, which is a very complicated process that finally results in the formation of sperm cells. During the process of moving to the epididymis, they go through several changes, the most significant of which are the acquisition of motility and the increase in the compression of the chromatin material^{5,6}. To determine the generation of sperm, sperm analysis is performed, which includes determining their motility, count, concentration, and morphology. It is believed that this test is able to accurately forecast a pregnancy that occurs naturally^{7,8}.

Inhibin B is a glycoprotein hormone produced by Sertoli cells. It has a major role in regulating the secretion of the FSH hormone, which is secreted by the pituitary gland via negative feedback⁸. High FSH levels can stimulate the production of inhibin B, which is associated with spermatogenesis^{9,10}. This hormone provides accurate prediction of spermatogenesis by working with FSH, as the former's role is to regulate the latter's levels. Inhibin B indicates the function of Sertoli cells only^{11,12}. Infertility rates have increased recently, and this is due to the quality and composition of sperm, especially in the last ten years, as the rate of infertility among men has increased more than among women. The reason for the spike is the male factor of infertility, as it affects sperm formation, which leads to the appearance of acquired reproductive system defects, congenital malformations, glandular reproductive infections, endocrine disorders, and genetic immune factors¹³. Bacterial infections are among the most important factors affecting this¹⁴. The effect of microorganisms on sperm is indicated by several mechanisms, including increased levels of free oxygen radicals¹⁵, activation of autoimmunity against sperm and production of antisperm virulence factors¹⁶, excessive use of antibiotics¹⁷, sperm aggregation and clumping, and cellular morphological changes¹⁸. It has been found that bacteria belonging to the *Staphylococcus aureus* species are resistant to a wide variety of antibiotics¹⁹.

In addition to their rising resistance to antibiotics,

particularly resistance to methicillin and vancomycin, they are responsible for the spread of disease because they possess malignant genes that code for enzymes, toxins, and other components that are linked to cancer. This Gram-positive bacterium is one of the most common types, as it can be extracted from sperm samples. A decrease in sperm motility as well as clumping and aggregation of sperm are the results of studies that have demonstrated its prevalence in semen to a greater extent than other types²⁰.

Methodology

Study Settings and Population: This cross-sectional study, was conducted in the Department of Forensic Science, College of Science, Al-Karah University for Science, Baghdad, Iraq, during the period from April 2023 to August 2025 for two and half year. Adult men aged between (17 and 51 years) were registered in this study. Participants were selected from the rest based on routine semen analysis, where the sperm count was recorded and divided into three groups: a group with a Oligospermia count, a group with a normal sperm count, and a healthy group. The study also included registering patients with genital tract infections. Samples were collected from different locations at Yarmouk Teaching Hospital, private external laboratories, and reproductive health and infertility specialist clinics.

Semen Collection: Semen samples were obtained from all participants by masturbation after a period of abstinence from intercourse lasting 3 to 4 days. The samples were collected in sterile, dry containers with large and wide openings and incubated at a temperature of 37 degrees Celsius. Afterwards, the semen analysis was performed within one hour. The semen analysis was performed according to the procedures of the World Health Organization for 2010. A part of the sample was taken, and the chromatin dispersion was examined using a SCA-Scope (Micrototic) device of Spanish origin according to the approved kit (Gold Cytosperm kit). This method operates on the principle of chromatin material dispersion, as described in (source), using special dyes that stain the chromatin in the form of halos.

Serum Collection: Five ml of venous blood was collected from each participant and separated by centrifugation. The serum was transferred to special tubes, and the concentration of inhibin B in the serum was measured using a commercially available enzyme-linked immunosorbent assay

(ELISA) kit designed by Ansh-Lab according to the catalogue (LOT=121521) . The normal range for the age group was between 134.5 and 173.9 pg./ml.

Bacterial Culture: Semen samples were collected and diluted in sterile saline (1:10). One milliliter of each sample was centrifuged at 15,000 rpm for approximately 15 minutes. The sediment was suspended in 100 µm of sterile saline after removing the supernatant. The concentrated sediment cell pellets were placed on blood agar and mannitol agar. All media were incubated at 37°C for 24 hours. The colonies' growth on the agar was determined based on their size and color characteristics. Bacterial isolates were examined using several biochemical and physiological tests to determine their sex, and the species was identified using a Vitek 2 system (BioMérieux, France) according to the manufacturer's instructions.

Statistical analysis: Data were statistically analyzed using the program statistical analysis system –SAS (21). are compared the significant differences between the averages using the Duncan test (22) polynomial.

Ethical Consideration: The study design has been approved by the College of Science Research Ethics Committee at the Al-Karah University for Science (Ref. No.0923/0074, dated the 25th of September 2023).

Results

Initially, more than 100 samples (semen and blood) were collected from adult men. Their semen was examined routinely, and 90 samples were selected from among the participants, ranging in age from 17 years to 51 years. 30 patients (33.3%) were classified as having oligospermia, 30 patients (33.3%) as having normal sperm, and 30 individuals (33.3%) as being healthy. Semen parameters were analyzed in terms of chromatin dispersion according to the kit method approved by SCA-Scope (Gold Cyto Sperm DNA Kit). Based on the examination, sperm appeared in two different forms: the first was a fragmented DNA form, and the second was a non-fragmented form for all study groups, as shown in (Figure I).

The relationship between different types of diseases and sperm characteristics based on DNA fragmentation were recorded. The study groups were divided into three groups. The first group had a low sperm count, with an arithmetic mean for DNA fragmentation in sperm of 73.94 ± 3.95 , while the mean for non-fragmented sperm was 26.76 ± 4.03 , indicating a significant difference between the two cases. In the normal sperm group, the fragmented and Non fragmented sperm recorded were (55.56 ± 3.73) and (47.44 ± 3.65) , respectively, where there is no significant difference between the two cases. In the healthy sperm group,

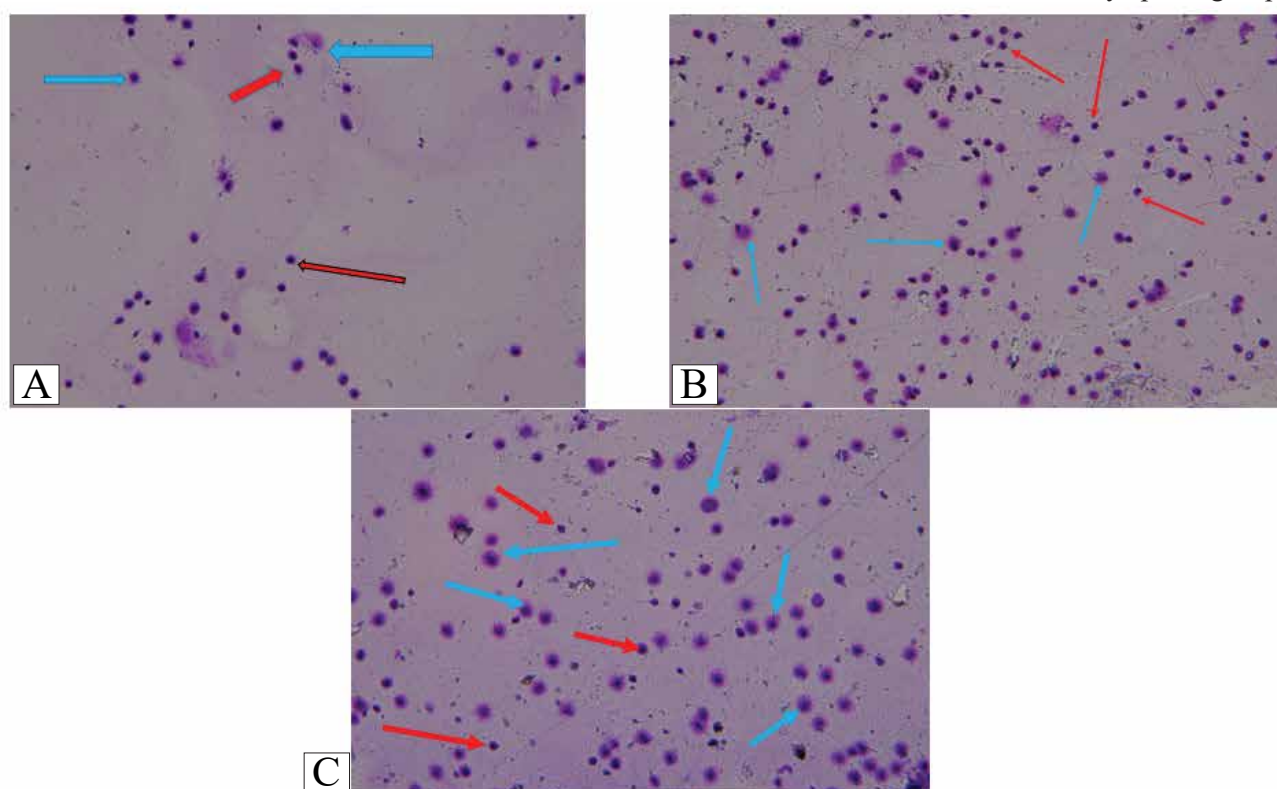


Figure I: Showing of Shape of sperm according to study group showing (Blue Arrow: not fragmented DNA, Red Arrow: fragmented DNA) A: Oligospermia group, B- Normal count sperm group, C- healthy group

the DNA fragmented and Non fragmented sperm recorded were 14.35 ± 1.69 and 85.03 ± 1.71 , respectively. There is a highly significant difference between the two cases. Regarding the significant differences among the three groups, a highly significant difference was found ($P < 0.01$) (Table 1).

Table 1: Relation Between the Types of Samples and Types of Pathogenic Case (Mean±Std. Error)

Type of Cases	Fragmented	Not Fragmented
Oligo	$73.94 \pm 3.95a$	$26.76 \pm 4.03c$
Normal	$55.56 \pm 3.73b$	$47.44 \pm 3.65b$
Healthy	$14.35 \pm 1.69c$	$85.03 \pm 1.71a$
Significant	**	**

The Role of Age in Sperm Pathology and Inhibin B Level Measurement: The age groups were divided into three categories. The first age group (17 to 27 years) showed a decrease in chromatin fragmentation in sperm compared to the other groups and an increase in normal-shaped sperm and inhibin B levels. The second age group (28 to 35 years) showed a lower mean percentage of sperm with chromatin fragmentation, while the percentage of normal sperm was high, as was the inhibin B level. The results for the third age group (37 to 51 years) were different. Sperm with genetic fragmentation were higher than in the other age groups, while the percentage of normal sperm was lower, and inhibin B levels were lower than in the other groups. There was a significant difference between the three age groups regarding chromatin fragmentation and its absence, but no significant difference was found in inhibin B levels, as shown in (Table 2).

Infections in the reproductive tract and their relationship to chromatin fragmentation in sperm and inhibin B levels in the blood: The number of semen samples infected with a bacterial infection (*Staphylococcus aureus*) was 46(40.0%), while the number of uninfected (healthy) samples was 54(60.0%), (Figure II).

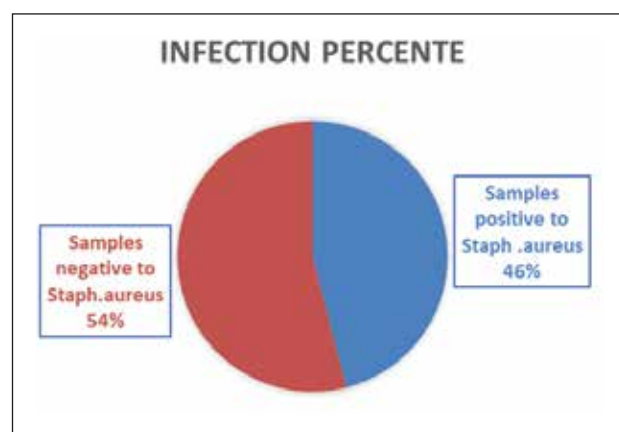


Figure II: showing of infection percent according to number of samples infected with *Staphylococcus aureus*



Figure III: Showing of growth bacteria (*Staphylococcus aureus*) in Mannitol agar isolated from Seminal fluid of human

Samples infected with bacterial infections exhibited an increase in chromatin fragmentation, with an arithmetic mean of 69.14 ± 3.85 , while these same samples demonstrated a decrease in the level of inhibin B hormone (53.90 ± 4.76). In contrast, healthy samples showed an increase in normal-shaped sperm and also an increase in inhibin B hormone, indicating a highly significant

Table 2: Distribution of Age groups according to Pathology of sperm and Inhibin B level in serum (Mean±Std. Error)

Age Group	Fragmented	Not Fragmented	Inhibin B
17 to 27 Years	$37.20 \pm 6.37b$	$63.64 \pm 6.19a$	$75.19 \pm 5.02a$
28 to 35 Years	$49.95 \pm 5.27ab$	$50.42 \pm 5.16ab$	$71.49 \pm 4.97a$
37 to 51 Years	$55.25 \pm 4.98a$	$46.62 \pm 4.95b$	$67.02 \pm 5.57a$
Significant	*	*	n.s.

Table 3: Relation between bacterial infection and Pathogenesis of sperm and Inhibin B in serum

Culture Staph Aureus	Fragmented	Not Fragmented	Inhibin B
Negative	33.82±3.66b	67.33±3.46a	82.50±3.01a
Positive	69.14± 3.85a	31.69± 3.87b	53.90±4.76b
Significant	**	**	**

difference between the healthy group and the group infected with *Staphylococcus aureus* bacteria (Table 3).

Discussion

Despite the high rate of male infertility, the precise mechanisms affecting such cases are numerous, including local injuries or infections in the reproductive tract, hormonal influences, and genetic and environmental factors, all of which play a major role in increasing the incidence of such cases. There are several causes of male infertility due to sperm DNA damage, foremost among them being abnormal chromatin packaging and sperm remodeling during spermatogenesis²³. Programmed cell death during epididymis maturation also plays a role²⁴, and oxidative stress has a pivotal role in infertility²⁵. Medications, physical therapy, radiation, bacterial infections, ageing, pollutants, and toxins also contribute to sperm DNA damage^{26,27}. Male infertility is also attributed to oxidative stress (ROS), which regulates the production of reactive oxygen species in seminal fluid, leading to an imbalance between oxygen species and antioxidants²⁵.

Studies on the development of infertility and the variation in sperm functions have proven that it is attributed to the presence of free radicals²⁸. It has been proven that oxidative stress and a decrease in the antioxidant capacity of sperm are related to the level of different types of free radicals²⁵. These radicals come from different sources, the most important of which are inflammation, infection, smoking, alcohol, radiation, toxic chemicals, male reproductive gland diseases, and varicocele²⁸. Genital infections are a common cause of male infertility. Mycoplasma, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are causes of genital tract infections that lead to an increase in nuclear fragmentation by interfering with the structure of DNA²⁷. This is attributed to the fact that they change the ratios of protamine 1 and 2²⁹. They also increase the production of ROS radicals, which leads to DNA damage³⁰. Age has always been a factor that negatively affects seminal fluid²⁷, as increasing age increases ROS types, lipid oxidation, and

mitochondrial stress, which is associated with ageing^{31,27}.

Studies have shown that men with normal sperm had higher DNA damage in older men, i.e., over 40 years of age, compared to males younger than 40 years of age³². This is consistent with a study that was conducted where the results were somewhat similar. Another study on 25,445 men who visit fertility doctors found that the father's age increases the percentage of DNA damage in sperm. Age is also an important factor, as the quality and quantity of sperm decrease with age in men³³. The rate of chromosomal abnormalities is associated with a decrease in sperm motility³², as the likelihood of damage to the DNA accumulated within the sperm increases, leading to increased damage³⁴. Studies have also proven the role of oxidative stress, which, when increased, weakens the mechanisms of DNA repair, leading to poor sperm quality³⁵. Another study conducted by³⁶ have proved that with age, the likelihood of damage to the DNA accumulated within the sperm increases, as this damage leads to the inability of the sperm to fertilise the eggs and may also increase the risk of genetic abnormalities in offspring.

These studies are also consistent with the current study, as older ages had a higher percentage of DNA damage in sperm. *Staphylococcus aureus* is a type of bacteria that causes diseases, particularly in the male reproductive tract. Numerous studies have revealed it to be the most frequently isolated type of bacteria from seminal fluid. Previous studies showed that 51.85% of seminal fluid samples were contaminated with *Staphylococcus aureus*, as indicated by study³⁷. Another study showed the presence of *Staphylococcus aureus* in 68.2% of seminal fluid³⁸. These studies support the current research, as the bacteria produce a protein with a molecular weight of 20 kDa, which was purified by study³⁹ and named SIF. This protein has the ability to completely immobilise sperm at a concentration of 150 mg/ml, while 200 mg/ml is the sperm-lethal concentration. Studies revealed pathogenic bacterial cultures in 48% of semen samples, with the most Gram-positive samples being *Staphylococcus aureus*. These results support the current study and are consistent with

previous studies conducted by⁴⁰ and⁴¹.

Thus, these results are consistent with the results of the current study, as the latter is an important bacterium in reproductive tract infections⁴². The explanation for this case is that after the bacteria enter, they recognize TLR-like receptors, which are pathogen recognition receptors, where they begin a series of inflammatory signals in the cells of the testis, epididymis, and areas of the male reproductive system⁴³. This response contributes to tissue damage and causes sperm defects that lead to infertility^{43,44}. Studies have shown a significant increase in sperm DNA fragmentation (SDF) in seminal fluid samples infected with *Staphylococcus bacteria*. This explains the effect of bacterial infection on sperm DNA integrity⁴⁵. Studies have shown a clear correlation between DNA fragmentation levels and bacterial factors in seminal fluid, indicating the presence of oxidative stress and inflammation in seminal fluid, which in turn leads to DNA damage⁴⁶. Numerous studies have also shown that infertile individuals have a higher percentage of SDF compared to fertile individuals^{47,48}.

This is consistent with the findings and is attributed to reactive oxygen species (ROS), which increase in their levels in the presence of bacteria⁴⁹. The increase in ROS is a result of the stimulation of white blood cells, which in turn affects DNA. This is related to the fact that white blood cells and their increase are associated with the presence of infection⁵⁰. Studies have shown that inhibin B levels in azoospermia are much lower than in men with normal sperm and oligospermia. It has also been shown that inhibin B is produced by Sertoli cells in response to FSH, and that inhibition of this hormone on spermatogenesis leads to decreased production of inhibin B, which fuels spermatogenesis disorder, as confirmed by⁵¹.

Other studies have shown that serum inhibin B concentration was much lower in men with failure of spermatogenesis, such as those with arrested maturation⁵¹. This suggests that serum inhibin B may be a reliable surrogate marker of spermatogenesis activity in men with oligospermia. These results are consistent with previous studies that have confirmed the role of inhibin B in assessing male infertility⁵²⁻⁵³. The strengths of this study lie in evaluating both the fragmentation of sperm nuclear material and its relationship to the inhibin B hormone, as well as bacterial infection, which allows for the evaluation and identification of the causes of weak sperm production and its normal composition. It provided a pathophysiological explanation for male infertility. Moreover, measuring the percentage of bacterial

infection reinforced the clinical importance affecting sperm physiology. Although the study sample does not exceed one hundred samples, it is possible to rely on the study as a starting point for finding the relationship of the inhibin B hormone and bacterial infection and its effect on the nature of sperm. In the end, such studies need comprehensive expansion, an increase in study samples, and further investigation.

Conclusion

This study demonstrated a strong correlation between blood levels of inhibin B and deterioration in semen quality, specifically sperm nucleic acid fragmentation. It also highlighted the significant role of *Staphylococcus aureus*, a common cause of genital tract infections, which was linked to sperm malformation and fragmentation, particularly of sperm DNA. This study underscores the importance of early diagnosis of these conditions using inhibin B blood levels and emphasizes the importance of treating infections with antibiotics to minimize damage and improve fertility, thus preventing sperm nucleic acid fragmentation in men. This work strengthens the effectiveness of treatment strategies for affected individuals.

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Conflict of interest:

The authors declared no conflict of interest.

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Authors' contributions

Conceptualization, methods and literature review: Hasan Ali Mohamed Aljezani; Statistical analysis and Data collection: Rafeef Yousif Rashid; Preparation of draft manuscript: Bairmani, M,S; Finalization of manuscript: Shahad Mohammed. All the authors approved the final manuscript.

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate The Institutional Review Board granted the study ethical approval. Since this was a prospective study, every study participant provided formal informed consent. Each method followed the appropriate rules and regulations.

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