

Original article

Bacterial infection, an upsurge of male infertility/subfertility: Evidence by semen analysis and sperm DNA fragmentation

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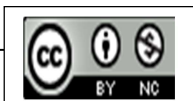
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Abstract

Background: Infection of the urogenital tract is considered as one of the major contributors to male infertility/subfertility. However, the effect of infection on semen parameters, especially on sperm DNA fragmentation, remains controversial. Therefore, in the present study effect of experimentally induced bacterial infection on several semen/sperm parameters and sperm DNA fragmentation was investigated.

Methods: Experimental infection of *Escherichia coli* and *Staphylococcus aureus* was induced in semen samples of healthy volunteers. Semen/sperm parameters including sperm DNA fragmentation were studied before and after experimental induction of infection.

Results: When various semen/sperm parameters were analyzed after experimental induction of *Escherichia coli* and *Staphylococcus aureus* infection, there was deterioration observed in these parameters. Upon comparison of various semen/sperm parameters after experimental induction of *Escherichia coli* and *Staphylococcus aureus* infection, there was significant deterioration observed in these parameters in *Escherichia coli* infection.

Conclusion: As observed in this study, bacterial pathogens like *Escherichia coli* and *Staphylococcus aureus* are capable of deteriorating the semen/sperm parameters. *Escherichia coli* produce a more damaging effect on sperm. The deleterious effect of bacterial infection on sperm quality may result in infertility/subfertility. Therefore, by considering the findings of this study, it can be concluded that infections should be considered as one of the parameters while investigating the patient with infertility/subfertility.

Key words: Bacterial infection, Sperm quality, Sperm DNA fragmentation

1. Introduction

Among all beautiful experiences in the life of an individual, parenthood is often considered the pinnacle. The journey of parenthood is filled with extreme joy, love and a sense of being complete and fulfilled. However, every individual is not fortunate enough to enjoy this happiness. Infertility is one of the major reasons for sorrow and unhappiness across the world. As per the World Health Organization (WHO), nearly 8-12% of couples belonging to the reproductive age group suffer from infertility worldwide.¹ Across the globe, more than 186 million people suffer from infertility, and the majority of them are residents of developing countries.²

As per the definition, "Infertility is a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse".¹ Infertility has several negative impacts on the life of an individual, as it affects nearly all aspects of life, including personal, social and emotional. It is also a reason for various psychiatric ailments, including depression, anxiety, distress and reduced quality of life. Additionally, infertility is also significantly associated with stigma and additional financial burden.³

Nearly 20-30% of cases of infertility are attributed to a male cause and 50% of overall cases of infertility are contributed by the male partner of couples facing problems with fertility.⁴ Infection of the urogenital tract is considered as one of the major contributors to male infertility/subfertility.⁵ However, the effect of infection on semen parameters, especially on sperm DNA fragmentation, remains controversial.⁶ Therefore, in the present study effect of experimentally induced bacterial infection on several semen/sperm parameters and sperm DNA fragmentation was investigated.

2. Materials and methods

Ethics approval: Institutional ethics committee clearance for the study has been obtained from Sri Sai College of Dental Surgery dated 14.09.2022 (Ref no. 03/SSCDS/IRB/2022).

i. Study design and population

This was a descriptive cross-sectional study, involving a total of 136 healthy male volunteers of the age group between 21-35 years. The selection of these volunteers was based on negative semen culture. Necessary consent was taken from the participants. Due efforts were taken to conceal the identity of the participants.

ii. Semen collection

Semen specimens were collected from the selected volunteers after 2-5 days of sexual abstinence. Oral instructions regarding the proper procedure for collecting the semen specimens were given to the volunteers before specimen collection. Additionally, written instruction was kept in a form of pamphlet in the room used for volunteers for collecting the semen specimens. The volunteers were instructed first to empty their bladder (urinate) and then wash their hands with plain soap and water. After this, volunteers were advised to wash their genital area thoroughly with water. Finally, the semen specimen was collected in a sterile, wide mouthed, non-toxic, container. The semen analysis of the volunteers was performed as per the procedures outlined in the latest edition of the World Health Organization (WHO) manual for the examination and processing of human semen.⁷ The parameters of semen/sperm analyzed included volume and pH of semen, liquefaction time of semen, and sperm concentration and motility.

iii. Semen culture

The loopful of semen specimen was aseptically inoculated onto the surface of blood agar, MacConkey's agar and chocolate agar. These inoculated plates were incubated aerobically at 37°C for 48 hours in an incubator. The plates were observed for the presence of bacterial growth. If any growth was observed, the semen specimen of these participants was excluded from the study. As mentioned in an earlier part of this section, the absence of infection, evident by negative culture was the criteria for selection of participants in the study.

iv. Detection of Sperm DNA fragmentation (SDF)

In the present study SDF was done by method is based on the Sperm Chromatin Dispersion (SCD). In this method a characteristic halo that is formed when nuclear proteins are removed after acid denaturation. In brief, the following steps were employed: i. Intact unfixed spermatozoa (fresh, frozen/unthawed, diluted or neat samples) were immersed in an inert agarose micro gel on a pretreated slide. ii. It was followed by incubation in an acid unwinding solution which transforms the DNA breaks into single-stranded DNA. iii. This preparation is then added to a lysing solution that breaks bonds between sperm DNA and surrounding proteins and clear nuclear proteins around the DNA. iv. Finally, the spermatozoa were stained and observed by bright-field microscopy (Make: Magnus, Model: MLX-B Plus). v. The sperm cells with normal DNA demonstrate big halos (a circle of light) in the nucleus whereas the ones with fragmented DNA appear with small or no halos.

v. Experimental induction of infection

The semen sample was divided into 3 equal portions. The 1st part was mixed with sterile peptone water and kept in an incubator at 37°C and it was used as a control. The 2nd part was mixed with peptone water, having 10⁶ Colony Forming Units (CFUs) of *Staphylococcus aureus* (isolated from a male with a urogenital tract infection), and kept in an incubator at 37°C. The 3rd part was mixed with peptone water having 10⁶ CFUs of *Escherichia coli* (isolated from a male with a urogenital tract infection) culture and kept in an incubator at 37°C. After 8 hours time interval control and test samples were examined for motility, viability, pH and SDF. Results were noted and compared with control and test samples to evaluate the effect of *S. aureus* and *E. coli* on sperm motility, viability, pH and SDF.

vi. Statistical analysis

The data obtained from the study was entered in the Microsoft[®] Excel spread sheet program for initial organization. t-test was performed to compare the means using SPSS version 24.0, IPM, USA.

3. Results

The mean age (years) of the participants was 27.8±4.2. The mean semen volume (mL) of participants was 3.84±1.2. All participants had normal pH i.e. 7.2 to 8. When the time for liquefaction of semen specimens was determined, the time of liquefaction for 114 specimens was within 30 minutes of collection, whilst 22 specimens liquefied within 1 hour of collection.

After 8 hours time interval control samples were examined, the mean sperm concentration ($\times 10^6/\text{mL}$) in the present study was 78.4 ± 4.3 . The mean ($\pm \text{SD}$) of total motile sperms was 44.6 ± 1.2 . The mean normal sperm morphology was 6.8 ± 1.3 . When sperm DNA fragmentation was assessed, the mean DNA fragmentation Index (DFI) was $20.8 \pm 1.2\%$ (Figure 1). The semen/sperm parameters of the participants were within normal ranges.

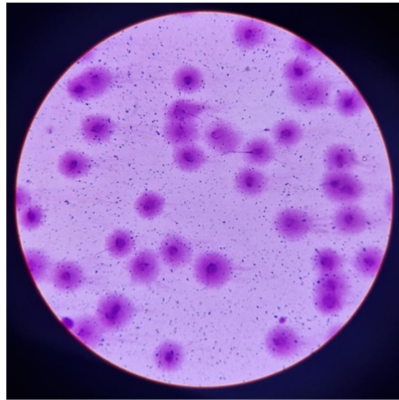


Figure 1. Microscopic observation of DNA fragmentation in control samples after 8 hours of incubation. The DNA fragmentation Index in normal range (DFI with $20.8 \pm 1.2\%$)

When various semen/sperm parameters were analyzed after 8 hours of experimental induction of *E. coli* and *S. aureus* infection there was significant deterioration observed in these parameters. The highest mean DNA fragmentation Index (DFI) of $46.6 \pm 1.1\%$ was recorded for *E. coli* infected semen/sperm samples (Figure 2). The *S. aureus* infected semen/sperm have recorded significantly lower mean DNA fragmentation Index (DFI) of $39.4 \pm 1.1\%$ (Table 1).

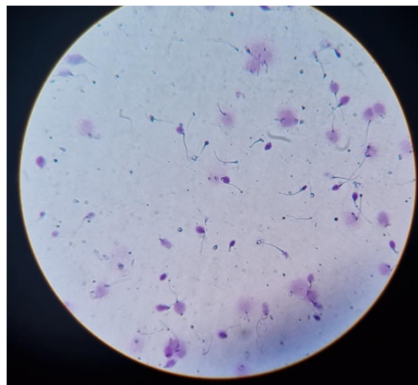


Figure 2. Microscopic observation of DNA fragmentation in semen samples induction with *E. coli* after 8 hours of incubation. Deterioration in DNA fragmentation Index (DFI with $46.6 \pm 1.1\%$)

Table 1. Comparison various semen/sperm parameters after experimental induction of *E. coli* and *S. aureus* infection

Parameter	Before inoculation (Mean $\pm$ SD)	<i>E. coli</i> infection (Mean $\pm$ SD)	<i>S. aureus</i> infection (Mean $\pm$ SD)	T test P value
DNA fragmentation index (DFI) %	20.8 ± 1.2	46.6 ± 1.1	39.4 ± 1.1	$<0.01^{**}$
Sperm concentration ($\times 10^6/\text{mL}$)	78.4 ± 4.3	40.3 ± 8.3	51.3 ± 2.1	$<0.01^{**}$
Total sperm motility (Mean $\pm$ SD) (%)	44.6 ± 1.2	21.6 ± 1.4	34.4 ± 1.4	$<0.01^{**}$
Normal sperm morphology	6.8 ± 1.3	2.6 ± 1.1	3.1 ± 1.2	$<0.01^{**}$

4. Discussion

Male infertility/subfertility can be attributed to various conditions like complete absence of sperm (azoospermia), low numbers of sperm (oligospermia), poor sperm motility (asthenozoospermia), abnormal sperm morphology (teratozoospermia) and other abnormalities that diminish the ability of sperm to fertilize an egg. Some of these factors are intrinsic, while others may be acquired.⁸ Urogenital tract infection is one of the acquired causes of male infertility/subfertility. These infections are of concern in nearly 8 to 35% of patients with compromised fertility. Several bacterial pathogens are implicated in reducing the fertility in males. These include *E. coli*, *S. aureus*, *Ureaplasma urealyticum*, *Chlamydia trachomatis*, *Mycoplasma*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Staphylococcus saprophyticus* and *Brucella* spp.⁹

There are many mechanisms postulated for infertility/subfertility induced by bacterial infections. These include destruction and alteration of function either directly by pathogens or indirectly via their products and/or inflammatory responses that are mediated through pro-inflammatory cytokines or reactive oxygen species (ROS), dysfunction of accessory glands of the urogenital system, obstructive inflammation and the host's immunity (cellular/humoral/both) against pathogens and its components. These events ultimately result in damage to the process of spermatogenesis, proliferation of anti-sperm autoantibodies and epigenetic changes.¹⁰ Although these mechanisms are proposed, there are only a few *in-vitro* studies exploring the exact effects exerted by the bacterial pathogens on the quality of semen/sperm. In the present study, *E. coli* and *S. aureus* infections were experimentally induced, by adding the organisms to the semen specimens collected from healthy participants. It was noted that sperm parameters like concentration, motility and morphology was significantly impacted by the bacterial infection.

In the present study, in addition to basic semen parameters, the sperm DNA fragmentation was also studied. The integrity of the sperm DNA is very essential for the process of fertilization and development of normal and healthy offspring. The integrity of sperm DNA affects the outcome of both natural conception and artificial reproductive techniques.¹¹ Loss of integrity or fragmentation is induced by both testicular and post-testicular mechanisms.¹¹ As observed in the present study, bacterial infections are also capable of inducing fragmentation in sperm DNA thereby, hampering the reproductive ability of an individual.

In the present study, when the comparison of semen/sperm parameters after experimental induction of *E. coli* and *S. aureus* infection was done, there was significant deterioration observed in these parameters in *E. coli* infection. Various mechanisms are considered responsible for deterioration of semen/sperm quality in infection by *E. coli*. These include adherence by *E. coli* with the help of pili, sperm agglutination due to a mannose-dependent effect, deranging the membrane of sperm, impairment of reactive oxygen species (ROS), impairment of sperm mitochondrial membrane potential, induction of scrambling of sperm lipid membrane, and toxin mediated cell mediated sperm death.¹²⁻¹⁴

5. Conclusion

As observed in this study, bacterial pathogens like *E. coli* and *S. aureus* are capable of deteriorating the semen/sperm parameters. *E. coli* produce a more damaging effect on sperm. The deleterious effect of bacterial infection on sperm quality may result in infertility/subfertility. Therefore, by considering the findings of this study, it can be concluded that infections should be considered as one of the parameters while investigating the patient with infertility/subfertility.

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