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Original article

Asymptomatic bacteriospermia: An occult threat to male fertility.

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Abstract

Background: *As per recent data, infertility affects between 48.5 million couples, amounting to 186 million individuals worldwide. Studies have documented that across the world, infertility is reported in approximately 30 million men, and nearly 50% of infertility cases can be attributed to the male partner of couples who have problems with conceiving. Although urogenital infection is one of the leading causes of male infertility/subfertility, the role of asymptomatic bacteriospermia remains largely unclear. The present study was conducted with the aim to determine the effect of asymptomatic bacteriospermia on various semen/sperm parameters.*

Methods: *Semen/sperm variables like semen volume, sperm count, motility and concentration, and sperm DNA fragmentation of 148 healthy volunteers were studied. Additionally semen culture was also performed. The semen/sperm parameters of participants showing presence of infection were compared with non-infected participants.*

Results: *Around 8.1% of participants were reported asymptomatic bacteriospermia and 4 cases for polymicrobial infection. Escherichia coli was found to be the predominant among the isolates screened. The semen volume and sperm motility were significantly varied among the participants (infected and uninfected) tested. Parameters like DNA fragmentation index and normal sperm morphology were highly altered.*

Conclusion: *Asymptomatic bacteriospermia was found to be linked with reduced semen volume and sperm motility. It also involved increasing the DNA destruction and abnormal sperm morphology. These findings imply that bacteria present in the semen may not show the symptoms, but they negatively impact male fertility potential. Hence, routine screening might be beneficial to avoid further complications.*

Keywords: *Asymptomatic bacteriospermia, Semen, Infertility, Subfertility, Escherichia coli, Male infertility*

1. Introduction

In the life of any living being, fertility has immense significance, as it is an important part of the normal lifecycle. Fertility makes the family complete and is extremely essential to establish one's identity in the society. However, all individuals are not fortunate to have this bliss of nature. According to recent data published by the World Health Organization in 2023, approximately 17.5% of the total global population (accounting roughly for 1 in 6 individuals) suffers from infertility.[1] Both infertility and its treatment can negatively affect every single aspect of an individual's life and manifests to as psychological-emotional disorders or consequences, including turmoil, frustration, depression, anxiety, hopelessness, guilt, and feelings of worthlessness in life.[2] Although it is an established fact that 40% of infertility cases are due to male factors, 40% are attributed to females and 20% are due to both or unknown causes, in developing countries the childbearing inability/ infertility is almost always attributed only to "woman", and women are mostly blamed for infertility even though at times the reason for infertility may be their male partner. [6] As per definition, male infertility is 'the male's inability to achieve pregnancy in a fertile female following at least 12 months of regular, unprotected sexual intercourse'.[3] Male infertility is a complex health condition, as it is caused and/or aggravated by a multitude of risk factors or causes. Aetiologies for male infertility may be hereditary or acquired.[4] Bacteriospermia, the condition characterized by the presence of bacteria in seminal fluid, is one of the most overlooked risk factors contributing to acquired infertility or subfertility.[5] Although, the role of symptomatic bacteriospermia in infertility/subfertility has been established, the effect of asymptomatic bacteriospermia on male fertility is largely unclear.[6] The present study was conducted with the aim to determine the effect of asymptomatic bacteriospermia on various semen/sperm parameters.

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).

Study design: A descriptive cross-sectional study was carried out at Sri Sai College of Dental Surgery with the sample size 148. Necessary consent was taken from the participants and due efforts were taken to conceal the identity of the participants.

Inclusion criteria: Healthy volunteers of age group between 20-35 years, persons with abstinence period of 3 days, men without urogenital tract infections were included in the study.

Exclusion criteria: Varicocele persons or persons with disorder in the male accessory sex gland secretion. Chronic smokers, Persons who had alcohol, caffeine, and drugs such as cocaine and marijuana two to five days before the collecting of sample, persons on any hormone medications, person on cancer treatment, men who work in the military, in high temperature, vigorous exercise or have jobs that include painting, driving, or repeated trauma to the pelvic area may be at risk for work-induced infertility, abstinence period > 5 days and <2 days, semen sample with liquefaction time more than 30 min were excluded from the study.

Sample collection: The participants were explained the procedure of sample collection and advised sexual abstinence for 3-5 days. Before collecting the specimens, the participants were asked to clean their hands with plain soap and wash their external genitalia with plain water. Semen specimens were collected in the sterile wide mouth poly-propylene container from the participants by masturbation in designated room close to the laboratory to avoid the contamination.

Semen analysis: Semen analysis was performed within 1 hour of sample collection as per the recent recommendation of the World Health Organization (WHO).[7] The variables of semen/sperms studied included volume (mL), concentration (10^6 mL^{-1}), total sperm number (10^6/ejaculate), motility (%), and morphology (%). These parameters were classified as per the 2021version of the WHO Laboratory Manual for the Examination and Processing of Human Semen.[7]

Bacterial culture: Semen specimens were inoculated onto the surface of Macconkey's agar, blood agar and Chocolate agar. The culture methods were incubated at 37°C for 48 hours in an incubator. The growth of bacteria, if any was identified up to species level by standard bacteriological protocol. Bacteriospermia was defined as $\geq 10^3$ colony-forming units (CFU)/mL.[6] Detection of sperm DNA fragmentation was done by Sperm Chromatin Dispersion method.

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 (in years) of the participants was 27.8±4.2 (range 21.6-38.9). The mean semen volume of participants (mL) was 3.84±1.2 (range 2.3 to 4.8). The mean sperm concentration (X10^6/ml) was 78.96±12.82 (10-200). The mean ±SD of total motile sperms (%) was 48.3±2.1 (range 34.5 to 100%). However, in 21 participants, the total motility of sperm was below >40%. The mean of rapidly progressive motility was 36.3±1.8% and the mean of progressively motile sperm was 38.4±1.4. The mean of non-motile sperm in this study was 18.6±1.9, which was within the normal limit. However, 16 participants had non-motile sperm count >20%. The mean normal sperm morphology (%) was 5.86±1.4 (range 3 to 7). The mean DNA fragmentation Index (DFI) was 16.41±1.8% (range 15-43%).

Upon semen culture, a total of 12 (8.1%) individuals showed the growth of bacteria. Out of these 12 specimens, 8 (66.7%) showed growth of single bacteria whereas 4 (33.4%) specimens showed growth of more than 1 bacterium (mixed infection). A total of 19 bacterial isolates were obtained from 12 culture positive semen samples. These included, 11 isolates of *Escherichia coli*, 4 isolates of *Staphylococcus aureus* and 3 isolates of *Klebsiella pneumoniae* and a single isolate was *Enterococcus* spp.

When semen/sperm variables were compared in normal participants and participants with infection, it was noted that semen volume and sperm motility were significantly altered in participants with infection. Parameters like DFI and normal sperm morphology were highly altered. Although sperm concentration was decreased in participants with infection, this difference was not statistically significant (Table 1).

Table 1. Semen/sperm variables in normal and infected participants

Variable	Normal Participants	Participants with infection	T test, *P* value
Total number of participants is 148	136	12	Not tested
DNA fragmentation index (DFI) %			
Mean ± SD	20.8 ± 1.2	32.15 ± 0.1	<0.01**
95% CI	15 - 30	28 - 40	
Semen volume (mL)			
Mean ± SD	3.4 ± 0.8	2.3 ± 0.1	<0.05*
95% CI	2.5 - 4.8	2.5 - 3.5	
Sperm concentration (x10^6/mL)			
Mean ± SD	78.4 ± 4.3	74.42 ± 8.2	>0.05
95% CI	10 - 200	10 - 200	
Total sperm motility (Mean ± SD) (%)	44.6 ± 1.2	28.3 ± 3.1	<0.05*
Normal sperm morphology			
Mean ± SD	6.8 ± 1.3	4.1 ± 1.1	<0.01**
95% CI	4 - 7	4 - 7	

*statistically significant, **statistically highly significant

4. Discussion

Urogenital infections and inflammation are established risk factors compromising the normal semen/sperm function and hence the male fertility.[8,9] Vast numbers of bacteria, fungi, viruses, and parasites are known to infect the urogenital system of man and induce a cascade of inflammatory responses that may lead to infertility/subfertility by interfering with the process of spermatogenesis.[10,11] Infection of the urogenital tract negatively affects the semen/sperm parameters like sperm vitality, and motility and morphology of sperm. However, the role of asymptomatic infection is not fully elicited.[6]

In the present study, when semen specimens from healthy were inoculated on suitable microbiological media, a total of 12 participants showed the growth of bacteria. *E. coli* was the predominant bacterial isolate. This member of Enterobacterales is increasingly implicated in infection of urogenital system. *E. coli* produces a deleterious impact on sperm quality by affecting its motility and impairing the functions of the acrosome.[12,13] *S. aureus* was the second most common isolate from the semen specimens. As compared to *E. coli*, till recent date the exact role of *S. aureus* is poorly investigated.[14] Aerobic Gram-positive cocci are reported to be present in nearly 50% of semen specimens of male partners in infertile couples.[15]

In the present study, it was revealed that there was a significant difference between semen/sperm variables of participants with a positive culture and participants with a negative culture. Participants with positive culture showed significantly higher numbers of immotile sperm, significantly less normal morphology sperm, and high DFI. Therefore, bacteriospermia, though without any symptoms has every potential to derange the semen/sperm parameters. The treatment of asymptomatic bacteriospermia with antimicrobials may benefit the patients seeking medical advice for infertility. Additionally, screening of the possibility of infection may also benefit the outcome of patients opting for assisted reproductive technology.

5. Conclusion

From the present study, it can be concluded that asymptomatic bacteriospermia does occur in healthy individuals. As these infections are not associated with any signs and symptoms, they often get neglected. Bacteriospermia, though asymptomatic has deleterious impact on semen/sperm parameters and may affect the outcome of natural or assisted reproduction. As compared to other, medications and procedures for treating infertility/subfertility, bacteriospermia can be treated with cost-effective and easily available antimicrobial agents. The role of semen culture while conducting laboratory workup for evaluating the causes of infertility/subfertility in males should not be overlooked.

6. Declaration

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