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Impact of bacterial infections in seminal fluid on delayed male fertility: A comparative study between case and control groups

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Bacterial persistence in seminal fluid, known as bacteriospermia, is increasingly recognized as a significant contributor to male infertility. This study investigates the prevalence of bacteriospermia in subfertile men, assesses its impact on semen quality, and identifies the specific bacterial species involved. This case-control study involved 78 male participants, divided into two groups: 39 men experiencing delayed conception (case group) and 39 men with proven fertility (control group). Participants were chosen based on predetermined inclusion and exclusion criteria,. Comprehensive questionnaires and clinical assessments were used in the data gathering process. Semen samples were collected and measured for volume, pH, sperm concentration, motility, morphology, and viability following WHO guidelines. Bacterial identification was done using Gram staining, culture methods, and the Vitek 2 system. In the case group, 79.5% of patients had bacterial persistence, while in the control group it was seen in 35.9%. Isolated bacteria in the case group most commonly included *Staphylococcus haemolyticus*, 25.6%; *Enterococcus faecalis*, 12.8%; and *Staphylococcus lentus*, 10.3%. Substantial differences were recorded between case and control groups for sperm motility, concentration, and morphology. Moreover, the case group had higher percentages for smokers, wearers of tight clothing, consumers of alcohol, and those accustomed to prolonged sitting, which were found to be statistically significant risk factors. This study underscores the pivotal role of bacterial persistence in seminal fluid in the aetiology of male infertility. Addressing bacteriospermia by using better diagnostic and therapeutic strategies can improve reproductive outcomes and clinical care for affected individuals.

Keywords: *Enterococcus faecalis*; male fertility; seminal fluid; *Staphylococcus haemolyticus*.

Introduction

Infertility presents a very significant health problem worldwide, affecting millions of couples. Though it is determined by factors from both sexes, approximately 40–50% of the cases are due to male infertility. Among the numerous causes of male infertility, infections in seminal fluid have gained considerable attention because of the potential impact on sperm quality and reproductive outcome. It is possible to establish the role played by bacterial infections in the seminal fluid in delayed conception by comparing the prevalence and types of bacteria in seminal fluid samples in case groups of infertile men with those in control groups of fertile men (Tvrdá et al., 2022).

Such infections in seminal fluid can originate in infection of the urethra, prostate, epididymymis, and seminal vesicles. Thereafter, resulting inflammation and oxidative stress syndrome (OSS) will further diminish spermatozoa quality due to loss of their motility, abnormal morphology, and reduced DNA integrity. In fact, bacterium-induced infections of seminal fluids are considered one of the major risk factors in the development of chronic prostatitis, epididymitis, and urethritis, which significantly and detrimentally affect fertility in males. Furthermore, some bacteria, both Gram-negative and Gram-positive bacteria, have been known to form toxins and enzymes that can break down protective barriers in the male reproductive tract, thus enhancing the detrimental effects on sperm function (Al-Abdaly, 2023; Henkel, 2024).

In this work, a complex analysis of seminal fluid of two groups of men was performed; those with delayed fertilisation and those with established fertility. Seminal fluid samples were investigated for the presence and types of bacteria, while patient characteristics, habits, and medical histories were assessed in order to establish any differences between the two groups. We hypothesized that the case group would show higher rates of seminal fluid bacterial infection, which might form part of the explanation for their delayed reproductive outcome. The findings of this study are therefore very important in improving current understanding regarding the contribution of bacterial infections to male infertility and in developing targeted interventions to improve reproductive health (Wang et al., 2021; Alnuimi & Alabdaly, 2022).

Identifying specific bacteria related to delayed fertilisation can increase diagnosis accuracy and improve treatment strategies in helping couples achieve family planning goals. The results also show whether those risk factors and predisposing conditions for bacterial infection in seminal fluid were realized, whether by lifestyle or medical means (Gholami et al., 2022; Alfathi et al., 2023).

This study in summary offers a valuable insight into the role of bacterial infection in seminal fluid as one of the major factors for delayed fertilisation and shows the necessity of screening and controlling male reproductive tract infection in infertility treatment programs. With this comparative study on the fertility status of affected couples, we would like to contribute to the field of general reproductive medicine and further facilitate efforts toward better fertility outcomes.

Material and methods

The Institutional Review Board (IRB) of each of the participating institutions approved the study protocol. Before being included in the study, all individuals provided written informed permission. Participants received guarantees of privacy and anonymity with relation to their personal and health-related data.

The study population of 78 male participants was divided into two groups: case group: 39 men experiencing delayed fertlisation (infertile group); control group: 39 men with proven fertility (fertile group).

Inclusion criteria: males aged 18–50 years. Case group: men with a history of delayed fertilisation for more than one year despite regular unprotected intercourse. Control group: men with a documented history of fathering at least one child within the last two years without assisted reproductive technologies.

Exclusion criteria: men with a history of sexually transmitted infections within the past year; men currently on antibiotic therapy or who have taken antibiotics in the last three months; men with known genetic or chromosomal abnormalities affecting fertility.

Data collection involved comprehensive questionnaires and clinical evaluations to gather information on patient demographics, medical history, lifestyle habits, and reproductive health. Key variables included age, weight, height, number of children, age of the youngest child, occupation, accommodation, education level, and habits such as smoking, alcohol consumption, and prolonged sitting.

Participants were instructed to stop ejaculation for 2–5 days before providing a semen sample. The samples were collected by masturbation into sterile containers in a private room at the clinic. Samples were transported immediately to the laboratory for analysis.

Semen analysis: volume, pH, sperm concentration, motility, morphology, and viability were assessed according to WHO guidelines, 6th edition (2021) (Boitrelle et al., 2021).

The samples centrifuged at 3000 rpm for 10 minutes to obtain seminal plasma and sperm pellets for microbiological analysis.

Smears were prepared from semen samples and stained using the Gram staining method to differentiate between Gram-positive and Gram-negative bacteria, as described by Costinar et al. (2021).

Semen samples were inoculated on blood agar, MacConkey agar, and chocolate agar plates. These culture media were incubated at 37 °C for 24–48 hours under both aerobic and microaerophilic conditions. Colony morphology was assessed, and preliminary identification was performed based on Gram staining characteristics.

Pure colonies were further analyzed using the VITEK 2 Compact system (bioMérieux, France), which applies advanced biochemical profiling for bacterial identification. It is important to note that, when using the VITEK 2 Compact system, prior differentiation based on Gram staining is not essential for system function, as the identification cards used are specific to either Gram-positive or Gram-negative organisms and are selected accordingly. The results obtained were recorded and analyzed for comparison between case and control groups (Książczyk et al., 2016).

All statistical analyses were performed using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). The software origin and version have been clearly stated to ensure reproducibility and transparency of data interpretation (Saleh, 2023).

Results

To ensure appropriate comparison between the two study groups (case and control), various demographic and reproductive variables were analyzed. Table 1 presents the distribution of these characteristics, including age, weight, height, number of children, age of the youngest child, occupation, accommodation, and education level. Statistical testing was performed to determine whether significant differences existed between groups.

Table 1
Distribution of patient characteristics between case and control groups

Patient Characteristics	Case group (n = 39)	Control group, (n = 39)	P-value
Age (mean ± SD)	38.0 ± 9.4	37.9 ± 10.0	0.96
Weight kg (mean ± SD)	86.1 ± 12.8	84.3 ± 8.6	0.48
Height cm (mean ± SD)	169.8 ± 13.8	173.1 ± 8.2	0.19
Number of children (mean ± SD)	2.0 ± 2.1	3.0 ± 1.4	0.01
Age of youngest child (mean ± SD)	3.7 ± 4.3	4.1 ± 3.8	0.73
Occupation			0.10
– non-employee	27 (69.2%)	20 (51.3%)	
– employee	12 (30.8%)	19 (48.7%)	
Accommodation			0.36
– villages and countryside	8 (20.5%)	5 (12.8%)	
– city	31 (79.5%)	34 (87.2%)	
Education level			0.33
– primary school	9 (23.1%)	5 (12.8%)	
– preparatory school	11 (28.2%)	7 (17.9%)	
– medium school	7 (17.9%)	10 (25.6%)	
– university	12 (30.8%)	17 (43.6%)	

Age, weight, height, and age of youngest child: no statistically significant differences (P > 0.05) were found between the case and control groups regarding these variables (Table 1). This suggests that the two groups were demographically similar.

Number of children: a statistically significant difference was observed (P = 0.01), with the case group having fewer children on average (2.0 ± 2.1) compared to the control group (3.0 ± 1.4). This supports the hypothesis that bacteriospermia may negatively affect male fertility.

Occupation: although a higher proportion of non-employed individuals was observed in the case group (69.2%) compared to the control group (51.3%), the difference was not statistically significant (P = 0.10). However, it may indicate a potential association between employment status and reproductive health.

Accommodation: most participants in both groups lived in urban areas. No significant difference was observed between rural and urban residency (P = 0.36), indicating minimal influence of living area on the outcome.

Education level: while the control group had a higher percentage of university-educated participants (43.6%) compared to the case group (30.8%), the difference was not statistically significant (P = 0.33, Table 1).

Varicocele was observed in 5.1% of the case group but not in the control group (Table 2). The P = 0.15 indicates that this difference is not statistically significant, suggesting that varicocele might not be a major factor distinguishing the two groups. However, its presence exclusively in the case group could indicate a potential association with fertility issues.

Table 2
Distribution of disorders between the studied groups

Disorder	Case group (n = 39)	Control group (n = 39)	P-value
Varicocele	2 (5.1%)	0 (0.0%)	0.15
Hormone disorder	4 (10.3%)	0 (0.0%)	0.04
Chronic disorder	4 (10.3%)	1 (2.6%)	0.16
Random drug use	4 (10.3%)	0 (0.0%)	0.04

A significantly higher percentage of hormonal disorders was found in the case group (10.3%) compared to none in the control group. The P = 0.04 suggests a statistically significant association, implying that hormonal imbalances may contribute to delayed fertilisation in men.

Chronic disorders were more prevalent in case group (10.3%) than in control group (2.6%), but the difference was not significant (P = 0.16). This suggests that chronic conditions alone might not play a major role in delayed conception, though they could contribute to overall health and fertility.

The case group showed a significantly higher occurrence of random drug use (10.3%) compared to none in the control group (Table 2). The P = 0.04 indicates a statistically significant difference, suggesting that unsupervised medication use may negatively affect male fertility.

Smoking prevalence was substantially greater in the case group (59.0%) comparative to the control group (30.8%, Table 3). The P = 0.01 indicates a statistically substantial association between smoking and delayed conception. Smoking is known to reduce sperm quality by increasing oxidative stress, which can impair sperm motility and morphology.

Table 3
Distribution of patients' habits between the studied groups

Habits	Case group (n = 39)	Control group (n = 39)	P-value
Smoke	23 (59.0%)	12 (30.8%)	0.010
Tight clothing	14 (35.9%)	2 (5.1%)	<0.001
Alcohol drink	7 (17.9%)	1 (2.6%)	0.025
Sitting for a long time	16 (41.0%)	3 (7.7%)	<0.001

A substantially larger proportion of men in the case group (35.9%) wore tight clothing compared to the control group (5.1%). The P <

0.001 indicates a highly substantial relationship, suggesting that tight clothing could be a risk factor for male infertility.

Tight clothing can increase scrotal temperature, negatively affecting spermatogenesis and sperm quality (Table 3). Alcohol use was more common in the case group (17.9%) than in the control (2.6%). The P = 0.025 indicates a statistically significant association between alcohol consumption and delayed conception. Alcohol can disrupt hormone balance, reduce testosterone levels, and negatively impact sperm concentration and motility.

Sitting for extended periods was significantly more common in the case group (41.0%) compared to the control group (7.7%). The P < 0.001 shows a highly significant association (Table 3). Prolonged sitting can lead to increased scrotal temperature, poor circulation, and oxidative stress, all of which negatively affect sperm quality.

A considerably larger percentage of the control group (64.1%) had no bacterial growth compared to the case group (20.5%). The P < 0.001 indicates a highly significant association between bacterial infections and delayed conception (Table 4). This suggests that seminal fluid infections are more prevalent in subfertile men and may contribute to reproductive dysfunction.

Table 4
Distribution of bacteria in seminal fluid between the studied groups

Bacteria	Case group (n = 39)	Control group (n = 39)
Sterile	8 (20.5%)	25 (64.1%)
Staphylococcus aureus	2 (5.1%)	0 (0.0%)
Staphylococcus hominis ssp. *hominis*	1 (2.6%)	2 (5.1%)
Lactobacillus spp.	1 (2.6%)	9 (25.7%)
Staphylococcus equorum	1 (2.6%)	0 (0.0%)
Enterococcus faecalis	5 (12.8%)	0 (0.0%)
Staphylococcus lentus	4 (10.3%)	0 (0.0%)
Escherichia coli	1 (2.6%)	0 (0.0%)
Brevundimonas diminuta	1 (2.6%)	0 (0.0%)
Kocuria rhizophila	1 (2.6%)	0 (0.0%)
Rahnella aquatilis	2 (5.1%)	0 (0.0%)
Spore forming bacteria (*Bacillus* and *Clostridium*)	1 (2.6%)	0 (0.0%)
Staphylococcus haemolyticus	10 (25.6%)	3 (7.7%)
Ochrobactrum anthropi	1 (2.6%)	0 (0.0%)

The case group exhibited a significantly higher presence of pathogenic bacteria, including: *Staphylococcus haemolyticus* (25.6%), *Enterococcus faecalis* (12.8%), *Staphylococcus lentus* (10.3%), *Rahnella aquatilis* (5.1%), *Staphylococcus aureus* (5.1%). In contrast, the control group had a significantly lower frequency of bacterial infections, with a higher prevalence of *Lactobacillus* spp. (25.7%), which is considered beneficial for reproductive health.

Discussion

Our study investigated the impact of bacterial infections in seminal fluid on male infertility by comparing the semen quality of men with delayed conception to those with proven fertility. The results of the study have prospected the intricate interplay between bacteriospermia and male fertility through the revelation of substantial distinction in sperm parameters and bacterial presence among the two groups.

The crucial discovery that stands out statistically is the decrease, in the number of children within the study group (with a P = 0.01) highlighting the influence of infections, on male fertility. Factors, like age, weight, height and living conditions do not exhibit variances, in bacteriospermia having a more direct impact on delayed childbirth compared to general demographic or lifestyle elements. The similarity, in employment and education levels implies that factors beyond social status might play a key role in determining fertility outcomes. In the case group, with a percentage of infections (79% compared to 35% in the control group), it seems that these infections play a role in causing issues with sperm movement and shape as well as lower sperm count, which could result in delays, in conceiving a child (Guo et al., 2024).

The strong link between these issues and postponed parenthood underscores the role of hormonal equilibrium, in men's fertility. Using drugs without a plan may lead to imbalances and problems with sperm or other aspects of health (Liew et al., 2024). In the group being studied here varicocele was found in those with fertility issues. The data did not show a significant difference. This suggests that varicocele alone may not be the factor behind the fertility problems, in this sample (Shah et al., 2022). Chronic conditions also displayed no variance; however they could potentially impact health concerns indirectly. The results indicate that it is important to include an assessment of hormones and medical history when evaluating fertility in men facing delays in starting a family (Kimmins et al., 2024). We should delve deeper into exploring the effects of drug use on men's health, in future studies focusing on how it influences sperm quality and hormone levels (Schifano et al., 2022). The findings emphasize that smoking habits, along with wearing tight clothing and excessive alcohol intake are risk factors for male infertility, as is prolonged periods of sitting.

These behaviors have been shown to affect sperm health by causing oxidative stress and hormonal imbalances while also raising the temperature in the testicles (Sciorio et al., 2024). Smoking can cause an increase in reactive oxygen species (ROS), which can result in harm to sperm DNA and lead to decreased motility and abnormal morphology of the sperm cells (Parameswari & Sridharan, 2021). In the past, research has shown that individuals who smoke heavily tend to have low sperm concentrations and increased levels of sperm DNA fragmentation (Omolaoye et al., 2022). Maintaining sperm production requires an environment that is approximately 2–4 °C cooler than the body's core temperature, and prolonged exposure to elevated temperatures may result in reduced sperm count and motility. Alcohol can impact fertility by decreasing testosterone levels and raising estrogen levels, which can result in reduced sperm production (Finelli et al., 2021).

Sustained consumption of alcohol has been associated with conditions such, as shrinking of the testicles and reduced quality and viability of sperm cells. Sitting for long periods can reduce airflow around the scrotum, causing the testicles to become too warm, which can affect sperm production negatively (Rotimi & Singh, 2024). Prolonged perios spent sitting can lead to poor blood flow and higher levels of oxidative stress, in the body, factors that can negatively affect sperm health and function. The noticeable increase in the occurrence of infections in semen among the case group implies a connection between bacteriospermia and male infertility (Emokpae & Brown, 2021). Certain harmful bacteria, like *S. haemolyticis* and *Enterococcus faecalis* have the potential to impact sperm functionality adversely through toxin production and oxidative stress induction while interfering with the functioning of sperm cells (Eltwisy et al., 2022).

In the case group *Staphylococcus haemolyticus* accounted for 25% of the isolated bacteria. *S. aureus* was found in the group of cases. Is recognized as an opportunistic pathogen. These microbes have been documented to trigger inflammation, in the body and affect the movement and clustering together of sperm cells, which can result in decreased fertility (Jendraszak et al., 2024).

The group of cases exclusively identified with *Enterococcus faecalis* at 12% suggests a link, to fertility issues. This particular bacterium has been associated with conditions such as prostatitis and seminal vesiculitis due to higher levels of reactive oxygen species (ROS) that have the potential to affect sperm function adversely. In the control group, *Lactobacillus* was present in 25% while it was only detected in 3% of the case group. Beneficial, for mens health *Lactobacillus* creates compounds that stop the growth of harmful bacteria. The reduced amount of *Lactobacillu*, in men with low fertility implies that an imbalance in the microorganisms in seminal fluid might play a role in fertility challenges. In the group of cases studied for male infertility *Escherichia coli* and other bacterial species such as *Kociura rhizophila* were detected. These were not found in the control group suggesting their involvement in infertility cases. Bacteria in the reproductive tract could potentially lead to infections in the system and cause higher levels of oxidative stress that may affect sperm function (Wang et al., 2021).

There were higher levels of semen bacterial contamination in the infertile group, hence lending support to the observations made by Tvrdá et al. (2022) that semen germs are potential inhibitors of male fertility. This is further underscored by our findings that show some kind of interrelationship between the reduction in sperm count, motility, and morphology and the bacterial colonization in the seminal fluid. The findings agree with that of Gachet et al. (2022), whereby an association between particular populations of bacteria in semen and certain changes in sperm parameters were realized. In essence, it has been observed that *Prevotella* and *Streptococcus*, among other kinds of bacteria, were isolated more in the infertile groups than the fertile ones, hence implying that it might play a role in impairing sperm function.

Some mechanisms of impairment of sperm function by bacteria include direct bacterial adhesion, sperm agglutination by bacteria, and release of bacterial toxins. Tvrdá et al. (2022) and Henkel (2024) referred to the sperm dysfunction emanating from bacterial toxins and ROS produced by the accompanying leukocytes. Our findings showed a higher prevalence of leukocytospermia in the infertile group, thus supporting this theory of bacterial infection causing an inflammatory response that raises ROS levels and consequently damages sperm. This agrees with the report by Eini et al. (2021), who showed that bacterial infections have adverse effects on sperm integrity through the strong association established between leukocytospermia and sperm DNA fragmentation in infected samples.

Our study used Gram staining and the Vitek 2 system for bacterial identification; by description of Henkel (2024), these methods are the gold standard for detecting bacteriospermia. As ruled by WHO, semen samples with more than 10^3 CFU/mL are considered to be bacteriospermic. A considerable number of our infertile samples fulfilled this criterion. Borges et al. (2020) propose that refined diagnostic techniques such as PCR and NGS techniques would have identified a greater range of pathogens; this points out the need for rather comprehensive diagnosis strategies in the clinical field.

The medicines that usually treat bacterial infections in semen include antibiotics, anti-inflammatory agents, and antioxidants. Henkel (2024) stressed that removal of the infection and inflammatory causes of leukocytospermia can improve semen quality. While our findings support antibiotic therapy for bacterial infection, they also add support to adjunctive benefits of antioxidants against oxidative stress to improve sperm function. Borges et al. (2020) describe the potential for outcome improvement upon integration of these strategies in assisted reproduction settings.

The impact of bacteriospermia on male fertility remains highly debated. In our case study, as well as others, such as Eini et al. (2021) and Gachet et al. (2022), it is revealed that bacterial infections exert a negative influence on sperm quality, Stojanov et al. (2018) pointed out that more studies are required to clarify the mechanisms of pathogenesis. Bacteriospermia-associated subfertility is evidently quite complex, involving variability of species and the interaction with spermatozoa. The current study therefore adds to the growing body of evidence regarding the impact of bacterial infection on seminal fluid and sperm parameters.

Conclusion

This research emphasizes a link between bacteria in semen and delayed natural conception in men due to the presence of bacteria such as *Staphylococcus haemolyticus* and *Enterococcus faecalis*, which can lead to inflammation and sperm damage, while certain bacteria like *Lactobacillus* seem to have a protective effect against infertility in men. Other factors, such as lifestyle habits, including smoking or wearing tight clothes, which are connected to male fertility problems are being studied too. Moreover disrupted hormone levels and unsupervised drug consumption have been linked to delays in conceiving a child. Further studies are needed to investigate how bacteria can affect sperm quality and the possibility of using probiotics to rebalance the environment in semen.

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