Patient Safety & Quality Improvement Journal
http://psj.mums.ac.ir
PSQI

Co-Occurrence of Seminal Bacteriospermia, Sperm Functional Impairment, and Anti-Sperm Antibody Positivity in Male Infertility: A Case-Control Study

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ARTICLEINFO

Article type:
Original Article

***Article History*:**
Received: 20 Aug 2025
Accepted: 13 Sep 2025

Keywords:
Anti-Sperm Immune Reaction; Bacteriospermia; Sperm Motility; Sperm Agglutination; Genital Diseases, Male

ABSTRACT

Introduction:
Bacterial infections and anti-sperm antibodies (ASA) are significant factors contributing to male infertility. This study examines the relationship between bacterial infection and ASA presence in the seminal fluid of infertile males.

Materials and Methods:
A case-control study was conducted from January to December 2023 at Dr. Nyaz Omer Rostam Clinic, Sulaymaniyah, Iraq. Eighty married men aged 20–40 years with abnormal semen analysis were enrolled and divided into two groups: 40 with confirmed bacterial infections (case group) and 40 without (control group). Semen analysis, bacterial culture, and MAR test for ASA were performed according to WHO 2021 guidelines.

Results:
The case group (CG) (n=40) exhibited significantly lower sperm motility (30.10% ± 12.05 vs. 44.48% ± 4.60; $p \leq 0.001$), viability (55.78% ± 8.04 vs. 63.95% ± 6.26; $p \leq 0.001$), and normal morphology (3.05 ± 1.44 vs. 4.78 ± 0.768; $p \leq 0.001$) compared to controls (n=40). Semen volume was higher in the CG (3.85 ± 1.69 mL vs. 3.18 ± 1.29 mL; $p = 0.05$). No significant differences were observed in sperm concentration or total sperm count. Bacterial cultures were positive in 40 (100%) of the CG, with Enterococcus faecalis detected in 10 (25%) and Staphylococcus haemolyticus in 8 (20%), while all controls (0%) were negative ($p \leq 0.001$). MAR test positivity (≥30%) was observed in all of the CG and in none of the controls (0%) ($p \leq 0.001$).

Conclusion:
The present study demonstrated that bacterial infections in the semen of infertile men are significantly associated with reduced sperm quality (especially motility, viability, and normal morphology.

► ***Please cite this paper as:***
Hamasallih Muhammed S, Omar Rostum N. Co-Occurrence of Seminal Bacteriospermia, Sperm Functional Impairment, and Anti-Sperm Antibody Positivity in Male Infertility: A Case-Control Study. Journal of Patient Safety and Quality Improvement. 2025; 13(4): 241-249. Doi: 10.22038/psj.2025.90631.1483

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Introduction

The World Health Organization (WHO) says that male infertility is when a man can't get a fertile woman pregnant after having unprotected sex for at least one year, with the male partner being solely responsible for about 20% of cases, and contributing to 30%-40% of all cases (1). Epidemiological studies show that infertility affects 8% to 12% of couples of reproductive age around the world (2).

There are many reasons why men can't have kids, which can be grouped by their cause: endocrine diseases (2–5%, like hypogonadism), problems with sperm transport (5%, like vasectomy), primary testicular defects (65–80%, often with no known cause), and idiopathic causes (10–20%, normal semen but no infertility) (1). Bacterial infections of the male genital tract and the presence of anti-sperm antibodies (ASA) in semen are two important factors that can severely affect a man's ability to have children (3).

Bacteriospermia is a common infection of the male genitourinary system that can make men infertile. These infections change the properties of semen for the worse, lowering the number of sperm, their ability to move, and the integrity of their shape, while also increasing the amount of DNA fragmentation in sperm (4, 5). Many men who are subfertile or infertile have been found to have pathogenic bacteria in their semen, such as Escherichia coli, Staphylococcus aureus, and Streptococcus species (6). These pathogens alter the seminal microenvironment, potentially compromising the blood testis barrier and permitting sperm antigens to elicit an autoimmune response, resulting in ASA production (7).

When sperm antigens break through immune privilege, the body makes anti-sperm antibodies. This can happen because of an infection, an injury, or inflammation. These antibodies can attach to different parts of sperm, making them less able to move, preventing them from becoming capacitated, and stopping them from interacting with eggs, which lowers the chances of fertilization (8). Clinical and laboratory data have shown that ASAs are more common in men who have genital tract infections, which supports the idea that infectious agents are involved in the immune system problems that cause male infertility (9, 10). Recent meta-analyses also show that men with bacterial infections of the reproductive tract are much more likely to have poor semen quality and develop ASAs (11).

It is very important to understand how bacterial infections cause the body to make anti-sperm antibodies in order to create targeted treatments that will help men with these infections become more fertile. Most of the time, current diagnostic methods look at these conditions separately, which could mean missing important links that could help doctors come up with better treatment plans. Therefore, the present study aimed to investigate the relationship between bacterial infection and the presence of ASA in the seminal fluid of infertile males.

Materials and Methods

1.1. Study design and setting

This case-control study was conducted between January 1, 2023, and December 31, 2023, at Dr. Nyaz Omer Rostam Clinic, a tertiary referral center for male infertility in Sulaymaniyah, Iraq. Ethical approval was obtained prior to commencement (Approval No. 132/245, dated 25 June 2025). The study adhered to STROBE guidelines for observational research.

1.2. Participants

Eighty married men aged 20 to 40 years, all presenting with abnormal semen parameters as defined by the WHO 2021 guidelines, were recruited from a fertility clinic for this study (12). Inclusion criteria were: (1) 20–40 year-old husbands of infertile couples; (2) documented abnormal semen parameters by WHO 2021 standards (volume <1.4 mL, concentration $<16\times10^6$/mL, total motility <42%, progressive motility <30%, vitality <54%, normal morphology <4%); (3) leukocyte count of $\geq1\times10^6$/mL or signs and symptoms of clinical urogenital infection (dysuria, urethral discharge, scrotal pain). And exclusion criteria were: (1) hormonal disorders (serum FSH >12 IU/L, testosterone <300 ng/dL); (2) anatomical abnormalities (varicocele Grade II–III,

cryptorchidism); (3) genetic conditions (Y-chromosome microdeletions, karyotype abnormalities); (4) current or recent (<4 weeks) antibiotic, anti-inflammatory, or antioxidant therapy; (5) history of reproductive tract surgery; (6) smoking or substance abuse. All participants provided written informed consent after explanation at length in either Kurdish or Arabic, as they preferred. A bilingual consent form was signed in the presence of an independent witness.

1.3. Sample Size

Sample size was calculated using G*Power 3.1.9.7 from pilot findings showing a 15% difference of sperm motility for inflected and not inflected groups (effect size d = 0.6; α = 0.05; power = 80%). The smallest sample per group required was 32. To account for possible sample attrition or sample contamination, 40 participants per group were recruited (total N = 80).

1.4. Semen Collection and Processing

Samples of semen were collected by masturbation in sterile, nontoxic plastic containers (Nunc™, Thermo Fisher Scientific, Cat. No. 361108) after 2–7 days of sexual abstinence. Collection was from a private temperature-controlled room (22–25°C) adjoining the laboratory. The genitalia and hands were cleaned with mild soap and distilled water prior to collection to rule out external contamination. Samples were transported to the laboratory in 10 minutes and held at 37°C for 30 minutes for complete liquefaction. The parameter analysis began for all of the samples within 60 minutes of collection in order to prevent parameter degradation.

1.5. Semen Analysis

The semen analysis was done according to WHO 2021 standards by two expert andrologists (inter-observer CV <5%) (1). Volume: Determined by graduated pipette (precision ±0.1 mL). pH: Determined using pH indicator strips (Merck, Cat. No. 109535).

Concentration and Motility: Assessed using a Makler® counting chamber (Sefi-Medical Instruments, Haifa, Israel) under phase-contrast microscopy (Olympus CX23, 200× magnification). At least 200 spermatozoa per sample were analyzed. Motility was categorized as progressive, non-progressive, or Immotile.

Vitality: Determined using the eosin-nigrosin supravital stain (Sigma-Aldrich, Cat. No. E4382). Two hundred spermatozoa were counted; unstained (viable) vs. pink-stained (non-viable).

Morphology: Defined by Diff-Quik® staining (Sysmex, Cat. No. 18610) of air-dried smears. Two hundred spermatozoa per sample analyzed by strict Kruger criteria at oil immersion (1000× magnification).

Leukocyte Count: Detected by the Endtz test (benzidine-H_2O_2 technique). Positivity results (brown sperm head) at 400×; gives results in leukocytes/mL.

1.6. Detection of Anti-Sperm

The direct mixed antiglobulin reaction (MAR) IgG test (FertiPro®, Beernem, Belgium, Cat. No. FP-MAR-100) was performed on liquefied semen within less than 1 hour of collection. Briefly: 10 µL of semen was placed in a mixture of 10 µL of IgG-coated latex beads and 10 µL of anti-IgG antiserum on a glass slide. After gentle rocking for 3 minutes, 100 motile spermatozoa were counted at 400× under phase-contrast microscopy. The percentage of spermatozoa attached to ≥3 beads was recorded. A value of ≥30% was considered clinically significant (positive for ASA).

1.7. Bacterial Culture and Identification

0.1 mL of each semen sample was inoculated onto four agar plates: (1) Blood agar (Oxoid, CM0055), (2) Chocolate agar (Oxoid, CM0033), (3) MacConkey agar (Oxoid, CM0007), and (4) Mannitol Salt agar (Oxoid, CM0009).

Blood, MacConkey, and Mannitol plates were incubated at 37°C for 24–48 hours under aerobic conditions.

The plates of chocolate agar were incubated at 37°C in 5% CO_2 for 48 hours.

Clinically significant bacterial growth was ≥1×10^3 CFU/mL. Identification of isolates was achieved on the VITEK® 2 Compact System (bioMérieux, Marcy-l'Étoile, France) by use of GN and GP identification cards, as directed by the manufacturer and by Bergey's Manual (10th ed.). Quality control strains E. coli ATCC 25922 and S. aureus ATCC 25923 were used every week.

1.8. Ethical Considerations

Ethical approval was obtained from the Ethics Committee of Sulaimani Polytechnic University (Approval Number: 132/245), dated 25 June 2025, under meeting No. 7 of the Vice President Office for Scientific Affairs & Postgraduate Studies. The study protocol was reviewed and approved by the Institutional Review Board of Sulaimani Polytechnic University and the Ethics Committee of Dr. Nyaz Omer Rostam Clinic. All research activities were conducted in accordance with the principles of the Declaration of Helsinki and local ethical regulations. Written informed consent was obtained from all participants after a detailed explanation of the study aims, methodology, potential risks, and benefits.

1.9. Statistical Analysis

Statistical Data were analyzed using IBM SPSS Statistics 28.0 (IBM Corp., Armonk, NY, USA). Normality was examined using Shapiro-Wilk test and Q-Q plots. Continuous variables (age, semen parameters) are reported as mean ± standard deviation and compared using independent samples t-tests. Categorical variables (bacterial species, MAR positivity) are reported as frequencies (%) and compared using Fisher's exact test. Pearson correlation coefficients were calculated to assess linear correlations between infection status and sperm parameters. Multivariable logistic regression was applied to define predictors of ASA positivity, adjusting for age, abstinence period, and leukocyte count. Statistical significance was set at $p < 0.05$ (two-tailed). Effect sizes (Cohen's d, Cramer's V) are reported for all significant results.

Results

Table 1 presents the age distribution and biological characteristics of sperm between the two groups. The mean (±SD) age was 32.83 ± 4.856 years in the CG and 33.30 ± 4.884 years in the control group. The mean (±SD) semen volume was 3.858 ± 1.694 mL in the CG and 3.185 ± 1.292 mL in the control group, showing a statistically significant difference between the two groups ($p \leq 0.05$). The mean (±SD) percentage of sperm motility (40% motility threshold) was 30.10 ± 12.049 in the CG and 44.48 ± 4.596 in the control group, indicating a highly significant statistical difference ($p \leq 0.001$). Regarding sperm viability, the mean (±SD) was 55.78 ± 8.04 in the CG and 63.95 ± 6.259 in the control group, which also showed a statistically significant difference ($p \leq 0.001$). Finally, the mean (±SD) percentage of normal sperm morphology was 3.05 ± 1.449 in the CG and 4.78 ± 0.768 in the control group, demonstrating a statistically significant difference between the groups ($p \leq 0.001$).

Table 1. Comparison of Age data and biological features of sperm Between Case and Control groups

Characteristics	Groups		P-value*
	CG (n=40)	Control group (n=40)	
Age	32.83 ± 4.856	33.30 ± 4.884	0.664
Volume ml.	3.858 ± 1.694	3.185 ± 1.292	0.05
Motality 40%	30.10 ± 12.049	44.48 ± 4.596	0.001
Viability	55.78 ± 8.04	63.95 ± 6.259	0.001
Normal morphology%	3.05 ± 1.449	4.78 ± 0.768	0.001
*P-value Based on Independent Samples t-test			

The mean (± standard deviation) sperm concentration (10^6/ml) per ejaculation was 72.700 ± 42.185 in the CG and 74.075 ± 33.023 in the control group. In the CG, 10 participants (25%) had a sperm concentration of ≤ 49×10^6/ml, while this was observed in 7 participants (17.5%) in the control group. A concentration between $50–99\times10^6$/ml was found in 24 participants (60%) from the CG and 20 participants (50%) from the control group. Additionally, a sperm concentration of ≥ 100×10^6/ml was reported in 6 participants (15%) in the CG and 13 participants (32.5%) in the control group.

Regarding the total sperm count (10^6/ejaculate), the mean (± standard deviation) was 230.502 ± 113.006 in the CG and 204.655 ± 78.697 in the control group. A total sperm count of ≤ 99×10^6/ejaculate was

identified in 4 participants (10%) in the CG and 3 participants (7.5%) in the control group. A total count between 100–199×10^6/ejaculate was observed in 14 participants (35%) from the CG and 17 participants (42.5%) from the control group. Finally, a total sperm count of ≥ 200×10^6/ejaculate was seen in 22 participants (55%) in the CG and 20 participants (50%) in the control group (Table 2).

Table 2. Comparison of Concentration and total sperm count of per ejaculation Between Case and Control groups

Characteristics		Groups		P-value*
		CG (n=40)	Control group (n=40)	
concentration (10*6/ml)		72.700 ± 42.185	74.075 ± 33.023	0.871
Concentration (10*6/ml) classification	≤ 49	10 (25%)	7 (17.5%)	0.204
	50-99	24 (60%)	20 (50%)	
	≥ 100	6 (15%)	13 (32.5%)	
Total count (10*6/ejaculate)		230.502 ± 113.006	204.655 ± 78.697	0.239
Total count(10*6/ejaculate) classification	≤ 99	4 (10%)	3 (7.5%)	0.794
	100-199	14 (35%)	17 (42.5%)	
	≥ 100	22 (55%)	20 (50%)	
*P-value Based on Independent Samples t-test				

The results of bacterial culture for both the case and control groups are summarized in Table 3. In the control group, bacterial cultures were negative for all participants. In contrast, in the CG, the culture results revealed the presence of several bacterial infections: Enterococcus faecalis was detected in 10 participants (25%); Staphylococcus haemolyticus in 8 participants (20%); Escherichia coli in 7 participants (17.5%); Staphylococcus epidermidis in 6 participants (15%); and coagulase-negative Staphylococcus in 3 participants (7.5%). Other less common bacterial infections were each identified in 1 participant (2.5%). The difference in bacterial culture results between the two groups was statistically significant (P ≤ 0.001) (Table 3).

Table 3. Comparison of Culture of bacterial infections Between Case and Control Groups

Characteristics		Groups		P-value*
		CG (n=40)	Control group (n=40)	
Culture of bacterial infections CFU>10*3/ml	Coagulase-negative Staphylococcus	1 (2.5%)	0	0.001
	Staphylococcus epidermidis	1 (2.5%)	0	
	Coagulase negative staphylococcus	3 (7.5%)	0	
	Enterococcus faecalis	10 (25%)	0	
	Escherichia coli	7 (17.5%)	0	
	No growth	0	40 (100%)	
	Staphylococcus aureus	1 (2.5%)	0	
	Staphylococcus epidermidis	6 (15%)	0	
	Staphylococcus haemolyticus	8 (20%)	0	
	Staphylococcus haemolyticus & Enterococcus faecalis	1 (2.5%)	0	
	Streptococcus mitis/Streptococcus oralis	1 (2.5%)	0	
	Streptococcus sanguinis	1 (2.5%)	0	
*P-value Based on Fisher's exact test				

The results of the MAR test ≥30% positive in both the case and control groups are presented in Table 4. In the control group, all participants tested negative for MAR ≥30% positive. In contrast, within the CG, MAR ≥30% positive results were observed as follows: a positivity rate of 95% in 5 participants (12.5%); a positivity rate of 38% in 4 participants (10%); and positivity rates of 55% and 65% each in 3 participants (7.5%). The difference in MAR ≥30% positive results between the two groups was statistically significant (P ≤ 0.001). Additional findings are summarized in Table 4.

Table 4. Comparison of MAR test >=30 % positive Between Case and Control groups.

Characteristics		Groups		P-value*
		CG (n=40)	Control group (n=40)	
MAR test >=30 % positive	Negative	0	40 (100%)	0.001
	Positive 68	2 (5%)	0	
	Positive 33	1 (2.5%)	0	
	Positive 35	2 (5%)	0	
	Positive 38	4 (10%)	0	
	Positive 40	1 (2.5%)	0	
	Positive 45	2 (5%)	0	
	Positive 46	1 (2.5%)	0	
	Positive 48	2 (5%)	0	
	Positive 50	1 (2.5%)	0	
	Positive 55	3 (7.5%)	0	
	Positive 60	2 (5%)	0	
	Positive 65	3 (7.5%)	0	
	Positive 70	2 (5%)	0	
	Positive 75	2 (5%)	0	
	Positive 80	2 (5%)	0	
	Positive 85	2 (5%)	0	
	Positive 90	2 (5%)	0	
	Positive 95	5 (12.5%)	0	
	Positive 99	1 (2.5%)	0	
*P-value Based on Fisher's exact test				

Discussion

This research examined the correlation between ASA in the seminal fluid of infertile men and bacterial infections, and how the latter affects the sperm quality and infertile status in men. The main results revealed that there was some statistical difference when the sperm parameters crucial were analyzed between the CG (infertile males) and the control group (fertile males) exhibiting significant differences in the semen volume, motility, viability, and normal morphology. It is important to note that the difference of concentration of sperm and total sperm count was not statistically significant between the two groupings.

Bacterial culture results revealed the exclusive and significant presence of bacterial infections in the CG, with a complete absence in the control group. The most common bacterial species isolated included Enterococcus faecalis, Staphylococcus haemolyticus, Escherichia coli, Staphylococcus epidermidis and coagulase-negative staphylococci. Moreover, the MSAR test, marker for ASA, was positive only in the CG and it was not found in control group, demonstrating a direct association between ASA and infertility.

Results of this study exceptionally showed that the sperm quality in the CG was significantly lower than that of control group in the infertile males. Significant reductions were observed in semen volume, sperm motility, viability, and normal morphology. Such findings are comparable with the existing studies that show the negative impact of the ASA and bacterial infections on the sperm quality. As an example, these results were confirmed by the study conducted by Leathersich and Hart (13), who demonstrated that ASA significantly impairs significantly lower the semen

volume, motility, viability and normal morphology. Similarly, Kuntareddi et al. (14) examined the production of ASAs, their specific binding sites on sperm, and their disruptive effects on key sperm functions—including motility, survival, acrosome reaction, and capacitation—demonstrating that ASAs can significantly impair sperm quality.

The present study also revealed that while sperm motility, viability, and morphology were markedly reduced in the infertile group, there was no significant difference in sperm concentration or total sperm count compared to the control group. This observation, in contrast to some studies that have reported a reduction in sperm concentration due to infections (15, 16), suggests that in the studied population, bacterial infections may primarily affect sperm function and structure post-production, rather than directly disrupting spermatogenesis. It means that the destruction of the effect of bacteria is more of the post-testicular nature, being realized due to direct attack of the sperm cell membrane, the introduction of apoptosis, or sperm motility blockage (17). This distinction in pathophysiology carries important clinical implications. If the primary issue is functional impairment of sperm, therapeutic approaches should focus on improving the quality of existing sperm through anti-inflammatory or antioxidant treatments, or by utilizing assisted reproductive techniques such as ICSI, which reduce the requirement for high sperm motility, even when sperm count is within the normal range. This also highlights that a "normal" sperm count does not necessarily exclude significant functional impairment due to infection, underscoring the necessity for comprehensive evaluation of functional sperm parameters.

Interaction of bacteria with the immune system (as they can trigger it to generate antibodies against sperm) will depress the quality of sperm and lower the chances of successful fertilization (18). Basically, many research studies have proved that infections of the male genital tract may profoundly interfere with these important sperm variables (19, 20). Specifically, E. coli and S. aureus are known for their ability to reduce motility and damage sperm morphology (21). Also related with low semen quality is Enterococcus faecalis in regard to semen concentration and morphology. These bacteria can damage the sperm in many ways; adhesion to the sperm itself, releasing cytotoxic toxins, or creating oxidative stress eat (22, 23).

The current study results on bacterial infection effect on male infertility support the earlier studies. Pai et al. (24) and Alzaidi et al. (25) have established that bacterial infection especially of the male reproductive system is a major factor contributing to infertile causes.

Anti-sperm antibodies (ASA) are recognized as a major factor in immunological infertility in males. A MAR test result of ≥30% is generally considered clinically significant (26, 27), and direct ASA testing methods, such as the MAR test, are preferred over serum-based assays (28). The main processes through which ASA is formed include interruption of the blood-testis barrier, which is typically a barrier that prevents access of sperms to immune system. In the present study, the prevalence of ASA in the seminal fluid of infertile males was significantly higher than in the control group (8, 29). The CG participants shared the positive results of MAR to varying degrees, whereas the control group did not show any positive MAR findings. These results concur with other studies which also indicated that there is a very strong increase in ASA levels in seminal fluids in infertile males (30, 31).

As the results clearly indicated, bacterial infections and ASA were observed simultaneously and exclusively in the infertile group. This concurrence strongly suggests a causal or facilitating relationship between these two factors in the pathogenesis of male infertility. Disorganization of the blood-testis barrier and inflammation (13), molecular mimicry (32), and synthesis of toxins and oxidative stress (33) of the bacteria might lead to ASA formation.

In this study, the use of the MAR test for ASA detection and bacterial culture for infection diagnosis represented validated and standard methodologies, thereby enhancing the reliability of the results. However, the relatively small sample size in

each group may have limited the ability to identify all potential correlations, particularly for less prevalent bacterial species, and may restrict the generalizability of the findings to larger and more diverse populations. Also, leukocytospermia, cytokines, and oxidative stress (ROS) as inflammatory mediators of pathologic pathway of infected sperm loss and ASA creation were not directly evaluated in the current study. The lack of such tests restricts the proper comprehension of the mechanisms underlying the process.

Conclusions

This study demonstrates that bacterial infections in the seminal fluid turn out to have relative relation to poor quality of sperms- their motility, viability as well as normal morphology and existence of ASA. This can be highlighted by the fact that these are the only factors that were found to be present in the infertile group and is thus responsible in the etiology of male infertility. Possible ways of these are direct damage of sperm by bacteria, generation of oxidative stress, and inflammatory reactions and ASA generation via molecular mimicry. This conclusion draws attention to the fact that male infertility involves such a complex of problems and necessitates comprehensive diagnostic evaluation to guide targeted therapeutic interventions.

Acknowledgments

We extend our heartfelt appreciation to all individuals whose time, dedication, and expertise were instrumental in the successful completion of this study.

Conflict of interest: All authors declare that they have no conflicts of interest related to this study.

Data availability: Upon reasonable request, the data from the research may be obtained from the corresponding author.

Authors' contributions: Each author made an equal contribution to this research work.

Funding: Not applicable

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