Original Article

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Impact of Microbial Infection on Sperm Parameters of Seminal Bacteria in Asymptomatic Subfertile Males

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Purpose: This study examined the effects of asymptomatic bacteriospermia on the semen quality of subfertile males. The types of bacteria and their antibiotic susceptibility were also analyzed.

Materials and Methods: Semen was collected and analyzed from 510 subfertile males. One hundred and seventy-nine males showed bacteriospermia, while 331 males did not. The bacterial species, sperm parameters, hormone levels, underlying disease, and lifestyle patterns were compared between the two study groups.

Results: The bacteriospermic males showed significantly higher rates of leukocytospermia (p=0.001) and deoxyribonucleic acid (DNA) fragmentation than the non-bacteriospermic males. Sperm motility was significantly lower in the bacteriospermic males than in non-bacteriospermic males. The most common seminal bacterial species were Prevotella bivia (P. bivia, 41.3%) and Ureaplasma urealyticum (U. urealyticum, 13.4%). U. parvum showed the highest recurrence rates (31.8%) three months after the initial antibiotic treatment. Regarding the sperm parameters of bacteriospermic males, the sperm concentration, total motility, progressive motility, leukocytospermia, and DNA fragmentation were improved significantly after the initial antibiotics treatment. Multivariate logistic regression analyses revealed P. bivia, U. urealyticum, and U. parvum to be associated with the decreased motility and increased DNA fragmentation of spermatozoa. P. bivia was also associated with a decreased sperm concentration (p=0.002) and vitality (p=0.013).

Conclusions: Bacteriospermia decreased the sperm concentration, motility, normal morphology, and vitality. P. bivia is the most commonly observed bacteria in subfertile males. Appropriate antibiotic therapy of seminal bacteria species had a strong positive impact on improving the semen parameters.

Keywords: Semen; Infection; Male infertility; Anti-bacterial agents

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INTRODUCTION

Male subfertility could result from multiple factors and represents the status of reduced fertility for a prolonged period (more than 12 months) of undesired non-conception. In contrast, the term is often used synonymously with infertility [1]. Male infertility affects 1 in 20 men and contributes to approximately half of all infertile cases [2]. The most commonly observed form of male subfertility is oligoasthenoteratozoospermia, which implies improper seminal profiles accompanying decreased sperm count and motility with increased abnormal morphology of spermatozoa [1]. Genitourinary tract infections (GUI) negatively influence the seminal quality, and approximately 6-44% of male infertility cases are related to GUI and bacteriospermia. In contrast, bacteriospermia is defined as the bacterial presence of ≥1,000 colony-forming units (CFU)/ml in human ejaculate [3]. Although some studies failed to prove a significant association between bacteriospermia and seminal inflammation [4,5], acute or chronic GUI might result in an inflammatory condition accompanying leukocytospermia, the abnormally high presence of leukocytes, which is generally defined as $>1\times10^6$ leukocytes in seminal fluid [6]. GUI may cause reduced spermatozoa function and the entire spermatogenesis process because of the direct spermatotoxic effect of bacteria, elevated oxidative stress with reactive oxygen species, and deoxyribonucleic acid (DNA) fragmentation of spermatozoa [6]. Lipopolysaccharide (LPS) is a major component of the Gram-negative bacterial cell wall. LPS reduces the intracellular cyclic-adenosine-monophosphate, leading to elevated reactive oxygen species (ROS) production and sperm membrane disruption [7]. DNA fragmentation induced by seminal bacterial infection may cause congenital defects and fetal spontaneous abortion, and previous studies presented some bacterial species, including Chlamydia trachomatis (C. trachomatis), Ureaplasma urealyticum (U. urealyticum) and Mycoplasma species, are associated with the DNA fragmentation of spermatozoa [8]. Bacterial infections can also result in sperm-bacterial interactions, leading to sperm agglutination. This sperm-bacterial agglutination causes the production of a biofilm that can accommodate more vulnerable surroundings for further bacterial colonization [9]. The impact of seminal bacteria on semen quality has not been fully exposed, and it is unclear if seminal bacterial infection in asymptomatic males is associated with male subfertility [10]. Moreover, the bacterial species-specific impacts on sperm parameters and related antibiotic treatment needs in asymptomatic males are controversial [11]. Thus, this study examined the effects of bacteriospermia on semen quality in subfertile males by analyzing the sperm parameters before and after antibiotic treatment. This study also evaluated the types of seminal bacteria and their antibiotic susceptibility.

MATERIALS AND METHODS

1. Patients

The study was a single-center retrospectively designed study of 510 subfertile males attending the Fertility Center of Bungang CHA Hospital from January 2022 to December 2022. A subfertile male was defined as a male who had unwanted non-conception for more than one year while continuing unprotected regular sexual intercourse with his female spouse. All study participants showed at least one impaired sperm parameter according to the initial semen test with reference to the World Health Organization 2010 criteria. The patients with symptomatic GUI, azoospermia, genetic disorders, anatomical abnormalities, and autoimmune disease-related subfertility were excluded. The sociobehavioural and clinical parameters of the study participants, including age, underlying diseases, previous history of genital surgery, previous hormone treatment history, smoking and alcohol consumption history, currently taking prescribed medications, and microscopic hematuria grade, were reviewed. Personal medical data collection and all clinical procedures were conducted according to recent relevant guidelines. The Ethics Committee of the CHA Medical University approved this study (registration number 2023-06-021). Signed informed consent was obtained from all study participants regarding the use of individual medical information.

2. Semen Collection and Analysis

All study participants underwent semen analysis and multiplex polymerase chain reaction (PCR) tests for bacteriospermia twice: on the first visit to the clinic and three months after the initial visit. At their first visit, all subfertile males included in this study were instructed to sustain their water intake at 2-3 liters/day to minimize the

possible GUI risk. Semen sample collections were undertaken at the clinic using sterile, non-toxic containers after three to five days of sexual abstinence. All study subjects urinated and washed their hands and external genitalia with disinfectant tissues twice to avoid unintended contamination during semen collection. All semen samples were analyzed 30 minutes after collection to maintain semen liquefaction. The analyzed semen parameters include the semen volume, sperm concentration, motility (total and progressive motility), morphology, vitality, and DNA fragmentation of spermatozoid. For DNA fragmentation analysis, the sperm chromatin dispersion was reviewed. The spermatozoa were classified as spermatozoa with fragmented DNA if they showed small halos or without halos or spermatozoa with cell degradation. Leukocytospermia was determined to be more than 1×10^6 leukocytes per ml of semen.

3. Analysis of Bacteriospermia and Antibiotic Susceptibility

The current study defined bacteriospermia as ≥1,000 CFU per ml of semen. The bacterial species were evaluated by extracting the microbial DNA using QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions. After microbial DNA extraction, bacterial strain specific multiplex real-time PCR (qPCR) was performed using NextGene™ STI Detection Kit (Ewon Reference Laboratory) to detect the following bacterial species: Neisseria gonorrhoeae, C. trachomatis, Ureaplasma parvum (U. parvum), U. urealyticum, Mycoplasma genitalium (M. genitalium), Mycoplasma hominis (M. hominis), Trichomonas vaginalis (T. vaginalis), Treponema pallidum (T. pallidum), Herpes simplex type-1 (HSV1), Herpes simplex type-2 (HSV2), Gardnerella vaginalis (G. vaginalis), Candida albicans (C. albicans), Escherichia coli (E. coli), Haemophilus ducreyi (H. ducreyi), Atopobium vaginae (A. vaginae), Streptococcus agalactiae (S. agalactiae), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumoniae (K. pneumoniae), Enterococcus faecalis (E. faecalis), Staphylococcus aureus (S. aureus), Bacteroides fragilis (B. fragilis), Mobiluncus curtisii (M. curtisii), Mobiluncus mulieris (M. mulieris), and Prevotella bivia (P. bivia). The identified bacterial species were tested for their antibiotic susceptibility. The antibiotic susceptibility of Ureaplasma and Mycoplasma species was evaluated using a conventional available liquid culture medium-based Mycoplasma IST-2 Kit (BioMerieux) according to the manufacturer's sensitivity interpretation guidelines. For the other bacterial species, minimal inhibitory concentration (MIC) was measured to determine the antibiotic susceptibility using the Clinical Laboratory Standards Institute disc diffusion method by measuring the annular radius of the inhibition zones for antimicrobial agents used. In contrast, the test results were interpreted and classified as sensitive, intermediate, and resistant. After the antibiotic susceptibility evaluation, each bacteriospermic male was treated with at least one commercially available antibiotic confirmed to be sensitive in preliminary susceptibility testing for the specific pathogen, even though the bacteriospermic males were asymptomatic regarding GUI.

4. Statistical Analysis

A Shapiro–Wilk normality test was performed to evaluate the normal distribution of the clinical variables. A student's t-test was used to compare the normally distributed variables, and a Mann–Whitney U-test was conducted for the variables with abnormal distribution. Univariate and multivariate logistic regression analyses with odds ratios were performed to evaluate the significant bacterial species for each semen parameter; p-values ≤0.05 were considered significant. All statistical data analyses were performed with SPSS version 24.0 (SPSS Inc.).

RESULTS

1. Baseline Socioclinical Characteristics

This study included 510 subfertile male patients. Table 1 lists the baseline socioclinical characteristics of the study subjects. Among these subfertile males, 331 males showed sterile semen according to qPCR tests, and the other 179 males were positive with at least one bacterial species, whereas 21 of them had ≥2 microbial presences (multi-microbial) in semen. All study subjects underwent qPCR tests for urinary pathogens to exclude a potential hindrance from bacteriuria to seminal microbial features. No pathologic bacterial presence was confirmed in all collected urine samples. Moreover, no patient accompanied lower urinary tract symptoms at the time of evaluation. The mean age of the subfertile males was 36.6 years, and the incidence of previous genital surgery history, including varicocelectomy, spermatocelectomy, and orchiopexy, was similar in

Table 1. Basic characteristics of the subfertile males (n=510)

	Total (n=510)	Bacteriospermic subfertile males (n=179)	Non-bacteriospermic subfertile males (n=331)	p-value[a)]
Age (y)	36.6±6.9 (29-44)	37.3±6.8 (30-44)	36.1±7.1 (29-43)	0.510
Underlying disease history				0.001
Hypertension	10 (2.0)	3 (1.7)	7 (2.1)	0.021
Chronic prostatitis	0 (0.0)	0 (0.0)	0 (0.0)	-
Cerebrovascular disease	2 (0.4)	0 (0.0)	2 (0.6)	0.056
Diabetes mellitus	24 (4.7)	19 (10.6)	5 (1.5)	<0.001
Presence of LUTS	0 (0.0)	0 (0.0)	0 (0.0)	-
Previous history of genital surgery				0.797
Spermatocelectomy	0 (0.0)	0 (0.0)	0 (0.0)	
Hydrocelectomy	9 (1.8)	3 (1.7)	6 (1.8)	
Orchiopexy				
Due to testicular torsion	0 (0.0)	0 (0.0)	0 (0.0)	
Due to testicular cryptorchidism	2 (0.4)	1 (0.6)	1 (0.3)	
Varicocelectomy	13 (2.5)	5 (2.8)	8 (2.4)	
Medical treatment prior to the first visit				
Clomiphene citrate	0 (0.0)	0 (0.0)	0 (0.0)	-
Testosterone replacement therapy	0 (0.0)	0 (0.0)	0 (0.0)	-
Initial hormone level				
FSH (mIU/ml)	5.2±1.5	5.1±1.4	5.4±0.9	0.151
LH (mIU/ml)	3.8±1.9	3.9±1.3	3.7±1.7	0.337
Testosterone (ng/ml)	4.8±1.4	5.0±1.2	4.7±1.1	0.242
Prolactin (ng/ml)	5.6±2.3	5.4±2.2	5.9±1.9	0.508
Semen parameters				
Volume (ml)	3.0±2.2	2.9±1.7	3.6±1.5	0.196
Sperm concentration (10^6/ml)	46.2±23.6	43.3±17.4	47.8±20.2	0.655
Total motility (%)	30.0±12.9	20.4±10.0	36.0±8.6	<0.001
Progressive motility (%)	18.1±7.1	11.1±5.3	22.5±4.7	<0.001
Leukocytospermia (10^6/ml)	0.8±2.1	2.8±1.9	0.3±0.2	0.001
DNA Fragmentation (%)	32.4±15.2	43.2±13.8	30.7±11.5	<0.001
Normal morphology (%)	2.1±2.3	1.5±1.2	2.4±1.5	0.239
Vitality (%)	53.0±9.7	51.7±6.1	55.9±8.4	0.575
Microscopic hematuria				<0.001
Overall	125 (24.5)	115 (64.2)	10 (3.0)	
Grade, RBCs/HPF				
0-3	100 (19.6)	93 (51.9)	7 (2.1)	
4-10	20 (3.9)	17 (9.5)	3 (0.9)	
11-25	5 (1.0)	5 (2.8)	0 (0.0)	
26-50	0 (0.0)	0 (0.0)	0 (0.0)	

the two study groups (p=0.797). Both study groups showed similar results regarding initial hormone levels, including follicular stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, and prolactin. Microscopic hematuria was observed more commonly in bacteriospermic males (64.2%) compared to non-bacteriospermic males (3.0%) (p<0.001). Bacteriospermic patients had a significantly larger number of patients (76.5%) with excessive alcohol use (>15 drinks/week) than non-bacteriospermic patients (30.5%) (p<0.001), but no significant differences in smoking history were observed between the two groups (p=0.576). Non-bacteriospermic patients had a significantly larger number of patients (24.2%) under 5-alpha reductase inhibitor (5ARI) treatment to manage androgenic alopecia compared with 16.2% of bacteriospermic patients (p=0.039).

2. Microbial Spectrum of Subfertile Males

Among the 179 bacteriospermic subfertile males, 88.3% of patients had a mono-microbial infection. In contrast, the other 11.7% of patients had a multi-microbial infection (Table 1, Fig. 1). The most frequently observed seminal pathogens were P. bivia (41.3%), U. urealyticum (13.4%), and U. parvum (13.4%). Other commonly identified bacterial species were E. faecalis (9.5%), S. agalactiae (8.9%), and M. genitalium (6.1%). M. curtisii, A. vaginae, and C. trachomatis showed seminal infection rates of 2.2%.

Table 1. Continued

	Total (n=510)	Bacteriospermic subfertile males (n=179)	Non-bacteriospermic subfertile males (n=331)	p-value[a]
Semen pathogen PCR analysis				
Pathogen spectrum				
Escherichia coli		0 (0.0)		
Chlamydia trachomatis		4 (2.2)		
Neisseria gonorrhoeae		0 (0.0)		
Ureaplasma parvum		22 (12.3)		
Ureaplasma urealyticum		24 (13.4)		
Mycoplasma genitalium		11 (6.1)		
Mycoplasma hominis		0 (0.0)		
Trichomonas vaginalis		0 (0.0)		
Gardnerella vaginalis		8 (4.5)		
Treponema pallidum		0 (0.0)		
Candida albicans		0 (0.0)		
Haemophilus ducreyi		0 (0.0)		
Atopobium vaginae		4 (2.2)		
Streptococcus agalactiae		16 (8.9)		
Prevotella bivia		74 (41.3)		
Pseudomonas aeruginosa		0 (0.0)		
Klebsiella pneumoniae		0 (0.0)		
Enterococcus faecalis		17 (9.5)		
Staphylococcus aureus		0 (0.0)		
HSV1		0 (0.0)		
HSV2		0 (0.0)		
Bacteroides fragilis		0 (0.0)		
Mobiluncus curtisii		4 (2.2)		
Mobiluncus mulieris		0 (0.0)		
Multi-microbial[b]		21 (11.7)		
Pathogen presence in semen, overall		179 (100.0)		-
Multi-microbial		21 (11.7)		
Mono-microbial		158 (88.3)		
Average alcohol consumption[c]				<0.001
0 drinks/wk	121 (23.7)	23 (12.8)	98 (29.6)	
1-15 drinks/wk	151 (29.6)	19 (10.6)	132 (39.9)	
>15 drinks/wk	238 (46.7)	137 (76.5)	101 (30.5)	
Smoking history				0.576
Never smoked	350 (68.6)	125 (69.8)	225 (68.0)	
Current smoker	97 (19.0)	34 (19.0)	63 (19.0)	
Previous smoker (current non-smoker)	63 (12.4)	20 (11.2)	43 (13.0)	
5-alpha reductase inhibitor treatment[d]	109 (21.4)	29 (16.2)	80 (24.2)	0.039

Values are presented as mean±standard deviation (range), number (%), or mean±standard deviation.
LUTS: lower urinary tract symptoms, FSH: follicle-stimulating hormone, LH: luteinizing hormone, DNA: deoxyribonucleic acid, RBCs/HPF: red blood cells/high-power field, PCR: polymerase chain reaction, HSV1: Herpes simplex type-1, HSV2: Herpes simplex type-2, −: not available.
[a]p-vlaues <0.05 are printed in bold characters. [b]Multi-microbial indicates equal or more than 2 bacterial species present in body fluid. [c]1 drink is equivalent to 1 glass of wine or 1 single spirit. [d]5-alpha reductase inhibitor was used for the treatment of androgenic alopecia.

3. Antibiotic Susceptibility of Seminal Pathogens

Table 2 lists the antibiotic susceptibility profiles of seminal pathogens confirmed from the subfertile males. All of the cases with P. bivia infection showed 100% sensitivity to metronidazole, ertapenem, piperacillin/tazobactam, and clindamycin, but ampicillin showed the highest resistance rate (31.1%) among the tested antibiotics. Among the microorganisms of the family Mycoplasmataceae, Ureaplasma species were completely susceptible to amikacin and doxycycline. On the other hand, one case of an M. genitalium infection (9.1%) was resistant to doxycycline. S. agalactiae were 100% sensitive to ampicillin, amoxicillin, ceftriaxone, ertapenem, and piperacillin/tazobactam, but E. faecalis were 100% resistant to ceftriaxone. All C. trachomatis cases showed the highest sensitivity (100%) with clarithromycin, amikacin, and doxycycline.

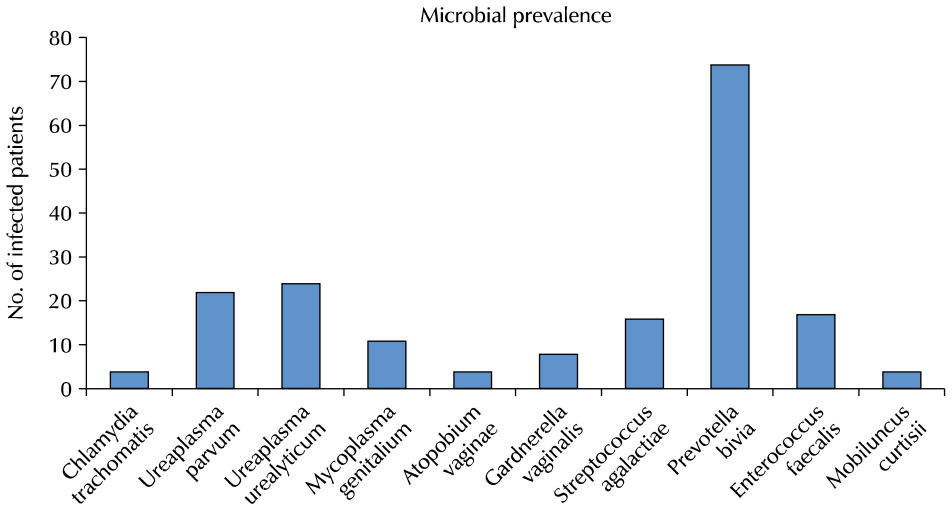

Fig. 1. Prevalence of microbial species in semen samples of subfertile males.

4. Effects of Bacterial Infections and Antibiotics Treatment on Semen Parameters

Table 3 lists the pre- and post-antibiotic treatment results for each bacterial species. All seminal bacterial infection cases were resolved after the initial antimicrobial treatment, but U. parvum presented the highest reinfection rate (31.8%) three months after the antimicrobial treatment. Regarding Ureaplasma species and M. genitalium cases, the total motility, progressive motility, leukocytospermia, and DNA fragmentation were improved significantly three months after the initial antibiotics treatment. After the initial antibiotics treatment of P. bivia, all sperm parameters were significantly improved except normal morphology, and no reinfection cases were observed during the three-month follow-up period. Leukocytospermia was improved significantly in all bacteriospermia cases after the initial antimicrobial treatment. The sperm vitality was improved after the antibiotic treatment in P. bivia and E. faecalis infection cases. The antimicrobial treatment increased the normal morphology of sperm only in the G. vaginalis and S. agalactiae infection cases. The eradication of seminal infection did not result in significant variations of the semen volume in all bacteriospermic cases.

5. Logistic Analysis for Predictors of Semen Parameters

Logistic regression analyses were undertaken to evaluate the predictors for specific semen parameters, and Table 4 lists the results. Regarding the decreased sperm concentration and vitality, P. bivia and E. faecalis were identified as independent predictors. There was no significant predictor identified in terms of a normal morphology. The seminal infection of U. parvum, U. urealyticum, M. genitalium, and P. bivia were associated with impaired sperm motility. S. agalactiae was associated with impaired normal morphology in univariate analysis, but it has lost independent predictor status in multivariate analysis. U. parvum, U. urealyticum, and P. bivia were associated with pathologically high DNA fragmentation of spermatozoa.

DISCUSSION

Male GUI and its adverse effect on sperm parameters have been discussed in many papers but remain controversial [12]. This study examined subfertile males with bacteriospermia and evaluated the prevalence of bacteriospermia among subfertile males and the negative effect of microbial infection of the male genital tract on the sperm parameters by analyzing the semen quality before and after antibiotics treatment. The results showed that 35.1% of subfertile males had a seminal bacterial infection, and adequate antibiotic treatments of specific seminal bacteria improved some sperm parameters. The current study showed bacteriospermic males had significantly more impaired sperm quality than non-bacteriospermia males in total and progressive sperm motility, DNA fragmentation, and normal morphology. Although no specific bacterial species were associated with impaired semen volume according to logistic regression analyses, the mean semen volume was increased in overall bacteriospermic males after antibiotics treatment regardless of infected bacterial types. This could be due to the increased daily water intake in study subjects [13] because all subfertile

Table 2. Antibiotic sensitivity of pathogens detected in urine or semen samples of subfertile males

	C. trachomatis (n=4)	U. parvum (n=22)	U. urealyticum (n=24)	M. genitalium (n=11)	G. vaginalis (n=8)	A. vaginae (n=4)	S. agalactiae (n=16)	P. bivia (n=74)	E. faecalis (n=17)	M. curtisii (n=4)
Ampicillin	N/A	N/A	N/A	N/A						
Sensitive	-	-	-	-	5 (62.5)	4 (100.0)	16 (100.0)	41 (55.4)	17 (100.0)	1 (25.0)
Intermediate	-	-	-	-	1 (12.5)	0 (0.0)	0 (0.0)	10 (13.5)	0 (0.0)	0 (0.0)
Resistant	-	-	-	-	2 (25.0)	0 (0.0)	0 (0.0)	23 (31.1)	0 (0.0)	3 (75.0)
Amoxicillin		N/A	N/A	N/A					N/A	N/A
Sensitive	2 (50.0)	-	-	-	5 (62.5)	4 (100.0)	16 (100.0)	45 (60.8)	-	-
Intermediate	0 (0.0)	-	-	-	1 (12.5)	0 (0.0)	0 (0.0)	8 (10.8)	-	-
Resistant	2 (50.0)	-	-	-	2 (25.0)	0 (0.0)	0 (0.0)	21 (28.4)	-	-
Ceftriaxone		N/A	N/A	N/A						
Sensitive	3 (75.0)	-	-	-	7 (87.5)	4 (100.0)	16 (100.0)	59 (79.7)	0 (0.0)	3 (75.0)
Intermediate	0 (0.0)	-	-	-	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.7)	0 (0.0)	0 (0.0)
Resistant	1 (25.0)	-	-	-	1 (12.5)	0 (0.0)	0 (0.0)	13 (17.6)	17 (100.0)	1 (25.0)
Clarithromycin							N/A	N/A	N/A	
Sensitive	4 (100.0)	10 (45.5)	12 (50.0)	5 (45.5)	6 (75.0)	4 (100.0)	-	-	-	4 (100.0)
Intermediate	0 (0.0)	1 (4.5)	0 (0.0)	1 (9.0)	0 (0.0)	0 (0.0)	-	-	-	0 (0.0)
Resistant	0 (0.0)	11 (50.0)	12 (50.0)	5 (45.5)	2 (25.0)	0 (0.0)	-	-	-	0 (0.0)
Clindamycin										
Sensitive	2 (50.0)	9 (40.9)	11 (45.8)	5 (45.5)	7 (87.5)	4 (100.0)	14 (87.5)	74 (100.0)	3 (17.6)	4 (100.0)
Intermediate	0 (0.0)	2 (9.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Resistant	2 (50.0)	11 (50.0)	14 (58.3)	6 (54.5)	1 (12.5)	0 (0.0)	2 (12.5)	0 (0.0)	14 (82.4)	0 (0.0)
Amikacin								N/A	N/A	N/A
Sensitive	4 (100.0)	22 (100.0)	24 (100.0)	11 (100.0)	8 (100.0)	4 (100.0)	16 (100.0)	-	-	-
Intermediate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-	-	-
Resistant	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-	-	-
Doxycycline								N/A		
Sensitive	4 (100.0)	22 (100.0)	24 (100.0)	10 (90.9)	1 (12.5)	4 (100.0)	2 (12.5)	-	5 (29.4)	2 (50.0)
Intermediate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (12.5)	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)
Resistant	0 (0.0)	0 (0.0)	0 (0.0)	1 (9.1)	6 (75.0)	0 (0.0)	14 (87.5)	-	12 (70.6)	2 (50.0)
Ciprofloxacin								N/A		N/A
Sensitive	3 (75.0)	10 (45.5)	10 (41.7)	4 (36.4)	3 (37.5)	3 (75.0)	15 (93.8)	-	0 (0.0)	-
Intermediate	0 (0.0)	0 (0.0)	2 (8.3)	3 (27.3)	0 (0.0)	0 (0.0)	0 (0.0)	-	0 (0.0)	-
Resistant	1 (25.0)	12 (54.5)	12 (50.0)	4 (36.4)	5 (62.5)	1 (25.0)	1 (6.2)	-	17 (100.0)	-
Metronidazole	N/A	N/A	N/A	N/A					N/A	
Sensitive	-	-	-	-	3 (37.5)	2 (50.0)	0 (0.0)	74 (100.0)	-	0 (0.0)
Intermediate	-	-	-	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	-	0 (0.0)
Resistant	-	-	-	-	5 (62.5)	2 (50.0)	16 (100.0)	0 (0.0)	-	4 (100.0)
Piperacillin/tazobactam	N/A	N/A	N/A	N/A		N/A				
Sensitive	-	-	-	-	8 (100.0)	-	16 (100.0)	74 (100.0)	17 (100.0)	4 (100.0)
Intermediate	-	-	-	-	0 (0.0)	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Resistant	-	-	-	-	0 (0.0)	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ertapenem	N/A	N/A	N/A	N/A	N/A	N/A				
Sensitive	-	-	-	-	-	-	16 (100.0)	74 (100.0)	17 (100.0)	4 (100.0)
Intermediate	-	-	-	-	-	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Resistant	-	-	-	-	-	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Values are presented as number (%).
C. trachomatis: Chlamydia trachomatis, U. parvum: Ureaplasma parvum, U. urealyticum: Ureaplasma urealyticum, M. genitalium: Mycoplasma genitalium, G. vaginalis: Gardnerella vaginalis, A. vaginae: Atopobium vaginae, GBS: Streptococcus agalactiae, P. bivia: Prevotella bivia, E. faecalis: Enterococcus faecalis, M. curtisii: Mobiluncus curtisii, N/A: not applicable, –: not available.

males included in the study were educated to maintain a water intake of 2-3 liters/day because drinking an adequate amount of water may prevent GUI [14].

Previous studies suggested the mechanism of impaired sperm quality induced by GUI with various bacterial species. In this study, the most commonly observed microorganism in bacteriospermic males was P. bivia, an anaerobic gram-negative microorganism suggested to be related to bacterial vaginosis and GUI. This result differed from previous studies, including Eini et al. [15] and Domes et al.

Table 3. Pre- and post-antibiotics treatment semen parameters in subfertile males with bacteriospermia[a)]

	U. parvum			U. urealyticum			M. genitalium		
	Pre-Tx (n=22)	Post-Tx (n=17)[d)]	p-value[e)]	Pre-T x (n=24)	Post-Tx (n=21)	p-value	Pre-Tx (n=11)	Post-Tx (n=10)	p-value
Semen parameters[b)]									
Volume (ml)	2.1±1.5	2.8±0.8	0.144	2.2±1.9	2.7±2.0	0.157	2.3±1.3	2.9±1.6	0.209
Sperm concentration (10^6/ml)	45.0±12.9	48.2±13.4	0.528	44.8±15.5	45.0±14.2	0.782	43.2±17.0	45.0±9.7	0.611
Total motility (%)	17.8±3.1	41.4±5.7	<0.001	17.5±6.9	47.0±10.1	<0.001	18.0±7.7	42.1±5.4	<0.001
Progressive motility (%)	7.9±4.3	31.7±5.9	<0.001	7.3±8.2	37.1±9.6	<0.001	9.2±5.6	32.9±6.2	<0.001
Leukocytospermia (10^6/ml)	3.0±1.4	0.4±0.4	<0.001	3.1±1.6	0.3±0.1	<0.001	3.0±1.2	0.5±0.2	<0.001
DNA fragmentation (%)	44.6±10.5	30.2±11.5	<0.001	45.7±8.6	31.4±5.3	<0.001	43.1±11.9	30.3±7.8	<0.001
Normal morphology (%)	1.1±0.5	1.1±0.9	0.560	1.1±1.3	1.2±1.1	0.617	1.5±0.8	1.5±1.0	0.770
Vitality (%)	47.9±6.5	50.4±7.3	0.284	45.2±8.4	47.5±10.3	0.589	46.5±6.1	49.0±11.4	0.331
Recurrence of bacterial infection[c)]	-	5 (31.8)		-	3 (12.5)		-	1 (9.1)	

	G. vaginalis			A. vaginae			S. agalactiae		
	Pre-Tx (n=8)	Post-Tx (n=8)	p-value	Pre-Tx (n=4)	Post-Tx (n=3)	p-value	Pre-Tx (n=16)	Post-Tx (n=16)	p-value
Semen parameters[b)]									
Volume (ml)	3.1±1.2	3.7±1.5	0.151	3.4±2.8	3.5±2.0	0.682	3.2±0.9	3.1±1.5	0.701
Sperm concentration (10^6/ml)	42.7±18.4	45.7±.10.5	0.553	49.9±17.2	52.9±10.3	0.604	39.1±10.9	42.1±10.6	0.728
Total motility (%)	22.1.±7.3	24.1±6.2	0.722	23.6±3.5	23.0±5.1	0.916	20.7±8.2	24.0±5.1	0.611
Progressive motility (%)	12.2±3.5	14.9±6.1	0.619	14.0±2.8	14.1±4.7	0.738	10.5±5.4	14.8±6.0	0.392
Leukocytospermia (10^6/ml)	2.2±1.7	0.2±0.1	0.001	2.5±1.3	0.6±0.2	0.001	3.5±1.5	0.3±0.1	<0.001
DNA fragmentation (%)	41.9±14.2	39.5±12.8	0.214	41.0±14.3	41.8±9.9	0.312	45.0±9.7	43.9±8.6	0.151
Normal morphology (%)	1.7±0.6	2.9±0.7	0.011	1.6±0.8	1.6±1.1	0.701	1.0±0.7	3.2±0.9	0.045
Vitality (%)	52.3±5.0	53.1±4.4	0.834	53.0±1.8	54.7±3.1	0.613	44.5±6.4	46.0±8.2	0.535
Recurrence of bacterial infection[c)]	-	0 (0.0)		-	1 (25.0)		-	0 (0.0)	

	P. bivia			E. faecalis			M. curtisii		
	Pre-Tx (n=74)	Post-Tx (n=74)	p-value	Pre-Tx (n=17)	Post-Tx (n=17)	p-value	Pre-Tx (n=4)	Post-Tx (n=3)	p-value
Semen parameters[b)]									
Volume (ml)	2.5±1.4	3.0±1.2	0.173	2.1±0.8	2.5±1.4	0.383	3.1±0.7	3.5±0.5	0.415
Sperm concentration (10^6/ml)	31.6±19.1	47.9±19.1	0.039	29.8±11.3	51.0±13.2	0.022	44.0±12.5	43.1±13.0	0.901
Total motility (%)	16.4±11.7	49.3±12.6	<0.001	23.7±4.4	40.8±13.4	<0.001	22.4±6.1	25.1±7.2	0.730
Progressive motility (%)	7.8±4.7	39.7±11.8	<0.001	13.6±3.9	31.3±5.1	<0.001	11.1±2.7	16.3±4.0	0.526
Leukocytospermia (10^6/ml)	3.6±1.1	0.3±0.2	<0.001	3.3±1.4	0.4±0.3	<0.001	2.6±1.5	0.3±0.3	<0.001
DNA fragmentation (%)	45.1±11.1	33.6±9.5	<0.001	43.0±12.4	41.7±18.0	0.001	41.6±7.8	41.4±10.8	0.796
Normal morphology (%)	1.8±0.5	2.0±1.3	0.127	1.5±1.1	1.6±0.9	0.636	1.6±0.4	1.6±0.9	0.659
Vitality (%)	48.7±7.2	58.0±4.1	0.029	49.9±5.3	59.2±8.4	0.018	44.9±5.7	47.4±9.2	0.627
Recurrence of bacterial infection[c)]	-	0 (0.0)		-	0 (0.0)		-	1 (25.0)	

Table 3. Continued

	C. trachomatis			Overall bacteriospermic cases		
	Pre-Tx (n=4)	Post-Tx (n=4)	p-value	Pre-Tx (n=179)	Post-Tx (n=168)	p-value
Semen parameters						
Volume (ml)	2.1±0.9	3.0±1.7	0.140	2.9±1.7	3.1±1.4	0.507
Sperm concentration (10^6/ml)	30.9±16.6	32.1±12.6	0.609	43.3±17.4	54.1±15.3	0.047
Total motility (%)	22.1±8.6	41.0±10.8	<0.001	20.4±10.0	35.8±8.2	0.031
Progressive motility (%)	13.3±4.8	32.3±6.1	<0.001	11.1±5.3	26.5±6.5	0.027
Leukocytospermia (10^6/ml)	4.0±0.9	0.5±0.2	<0.001	2.8±1.9	0.4±0.2	0.014
DNA fragmentation (%)	46.4±8.1	42.1±10.1	0.129	43.2±13.8	37.6±10.4	0.001
Normal morphology (%)	1.0±0.7	1.2±0.5	0.517	1.5±1.2	1.8±1.0	0.464
Vitality (%)	44.4±5.9	46.3±11.7	0.805	51.7±6.1	51.1±7.8	0.655
Recurrence of bacterial infection[c]	-	0 (0.0)			11 (6.1)	

Values are presented as mean±standard deviation or number (%).
U. parvum: Ureaplasma parvum, U. urealyticum: Ureaplasma urealyticum, M. genitalium: Mycoplasma genitalium, G. vaginalis: Gardnerella vaginalis, Tx: treatment, DNA: deoxyribonucleic acid, A. vaginae: Atopobium vaginae, GBS: Streptococcus agalactiae, P. bivia: Prevotella bivia, E. faecalis: Enterococcus faecalis, M. curtisii: Mobiluncus curtisii, C. trachomatis: Chlamydia trachomatis, –: not available.
[a]Genitourianry tract bacterial presence indicates bacterial infection confirmed in urine or semen samples. [b]Semen parameters are presented in mean±standard deviations. [c]Recurrence rate was evaluated at 3 months after the initial antibiotics treatment. [d]Post-antibiotics treatment semen analysis was performed on the patient who had no bacterial reinfection at 3 months after the initial antibiotics treatment. [e]p-vlaues <0.05 are printed in bold characters.

[16], who reported that E. faecalis was the most detected bacterial species in the semen of subfertile males. The differences in dominant seminal bacterial species must be derived from the variations of microbial distribution in the vaginal flora of the study subjects' female spouses. P. bivia frequently incorporates and stimulates the development of multi-microbial biofilms produced initially by GV that subsequently promote antibiotic resistance [17]. On the other hand, all P. bivia infection cases in this study showed metronidazole sensitivity, indicating no antibiotic-resistant biofilm formation might be accompanied. Moreover, a recent meta-analysis by Farahani et al. [18] reported the negative effect of Prevotella on sperm quality. The result was similar to the outcomes of the present study. The study results showed that all sperm parameters except normal morphology were improved significantly after the initial antibiotics treatment for P. bivia infection. Moreover, P. bivia was an independent predictor of sperm concentration, motility, vitality, and DNA fragmentation. Thus, a P. bivia infection in bacteriospermic subfertile males should be evaluated thoroughly, and eradicating P. bivia by proper antibiotic treatment, such as metronidazole, is recommended. Nevertheless, the significance of P. bivia as the dominant seminal microbial species in bacteriospermic subfertile males should be evaluated further with concurrent analysis of the vaginal flora of female spouses in a future study.

The current study investigated two Mycoplasma and Ureaplasma species, which are M. genitalium, M. hominis, U. parvum, and U. urealyticum, respectively. On the other hand, the influence on the sperm parameters by each Ureaplasma serovar was not analyzed separately. The negative role of Ureaplasma and Mycoplasma species on male infertility has been reported. Bai et al. [19] suggested infertile males carrying genitourinary infection by pathogenic bacterial species, including C. trachomatis, Ureaplasma, and Mycoplasma species, had a lower sperm concentration and higher semen leukocyte counts than pathogen-negative males. In this study, appropriate antibiotic treatment of U. parvum, U. urealyticum, and M. genitalium increased sperm motility and DNA fragmentation in subfertile males. Hence, the eradication of these bacterial species is recommended.

Non-bacteriospermic subfertile males of this study had significantly greater rates of 5ARI intake for androgenic alopecia treatment, which is similar to a previous study [20]

Table 4. Logistic analysis of bacterial species significantly influencing thespecific semen parameters of subfertile males

Semen parameter	Bacterial species	OR	95% CI	p-value[a)]	Bacterial species	OR	95% CI	p-value[a)]
Semen volume	None	-	-	-	None	-	-	-
Sperm concentration	PB	0.177	0.080-0.903	0.002	PB	0.122	0.019-0.757	0.030
	EF	0.110	0.045-0.691	0.011	EF	0.254	0.022-0.823	0.039
Motility	UP	0.119	0.009-0.618	0.021	UP	0.349	0.016-0.846	0.032
	UU	0.185	0.014-0.722	0.017	UU	0.245	0.119-0.531	0.040
	MG	0.103	0.034-0.545	0.033	MG	0.170	0.084-0.789	0.041
	PB	0.017	0.005-0.427	0.019	PB	0.085	0.011-0.332	0.027
	EF	0.072	0.005-0.167	0.035	EF	0.590	0.107-1.092	0.077
DNA fragmentation	UP	0.090	0.023-0.125	0.024	UP	0.151	0.083-0.199	0.036
	UU	0.078	0.012-0.153	0.012	UU	0.178	0.028-0.307	0.025
	MG	0.193	0.091-0.710	0.042	MG	0.406	0.091-1.185	0.106
	PB	0.088	0.060-0.109	0.005	PB	0.091	0.044-0.128	0.017
Normal morphology	GBS	0.091	0.080-0.096	0.036	GBS	0.011	0.031-1.202	0.105
Vitality	PB	0.122	0.056-0.451	0.013	PB	0.134	0.038-0.703	0.029
	EF	0.011	0.003-0.738	0.047	EF	0.023	0.010-0.512	0.047

OR: odds ratio, 95% CI: 95% confidence interval, PB: Prevotella bivia, EF: Enterococcus faecalis, UP: Ureaplasma parvum, UU: Ureaplasma urealyticum, MG: Mycoplasma genitalium, GBS: Streptococcus agalactiae.
[a)]p-vlaues <0.05 are printed in bold characters.

that reported that dutasteride decreases the risk of urinary tract infection in benign prostate hyperplasia patients during the four-year follow-up. Although the association between 5ARI use and reduced bacteriospermic risk was observed, the 5ARI treatment should be applied carefully in subfertile males because even low-dose-5ARI treatment for male pattern alopecia might impair the semen quality [21].

The bacteriospermic males of this study had higher rates of heavy alcohol consumption, whereas alcohol consumption caused abnormal morphological changes in sperm [22]. Therefore, all study subjects are recommended to quit or minimize (less than five drinks/week) alcohol consumption during the follow-up period.

This study had some limitations. Additional evaluations, including the semen ROS level, long-term semen quality variations, and vaginal flora of female spouses, were not performed because of the single-center retrospective nature with a relatively small number of samples. Thus, further studies with a larger sample size, long-term follow-up, and correlations with the female vaginal flora will be needed to confirm the current findings. Despite these limitations, this study showed that appropriate antibiotic therapy for asymptomatic bacteriospermia promotes significant improvements in the semen quality of subfertile males.

CONCLUSIONS

This study suggests that asymptomatic bacteriospermia in subfertile men has detrimental effects on sperm quality. Moreover, proper antibiotic therapy of those bacteria species, particularly P. bivia, improved the semen parameters. To the best of the authors' knowledge, this study is the first to present the clinical importance of P. bivia infections in male fertility. Therefore, routine analysis of bacteriospermia and subsequent antibiotic treatments should be performed in subfertile males.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

S.B.C. designed the study, analyzed and interpreted the clinical data, and wrote and revised the manuscript. T.J.K., T.H.K., S.R.L., D.S.P., and S.M.C. collected the clinical data and engaged in patient follow-up. D.H.L. analyzed and interpreted the clinical data. Y.D.Y. designed and supervised the project and revised the manuscript. All the listed authors have participated actively in the study. All authors read and approved the final manuscript.

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REFERENCES

1. Gnoth C, Godehardt E, Frank-Herrmann P, Friol K, Tigges J, Freundl G. Definition and prevalence of subfertility and infertility. Hum Reprod 2005;20:1144-7.
2. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol 2015;13:37.
3. Han H, Liu S, Zhou XG, Tian L, Zhang XD. Aetiology of obstructive azoospermia in Chinese infertility patients. Andrologia 2016;48:761-4.
4. Lackner J, Schatzl G, Horvath S, Kratzik C, Marberger M. Value of counting white blood cells (WBC) in semen samples to predict the presence of bacteria. Eur Urol 2006;49:148-52; discussion 152-3.
5. Punab M, Loivukene K, Kermes K, Mändar R. The limit of leucocytospermia from the microbiological viewpoint. Andrologia 2003;35:271-8.
6. Pajovic B, Radojevic N, Vukovic M, Stjepcevic A. Semen analysis before and after antibiotic treatment of asymptomatic Chlamydia- and Ureaplasma-related pyospermia. Andrologia 2013;45:266-71.
7. Zhang F, Dai J, Chen T. Role of Lactobacillus in female infertility via modulating sperm agglutination and immobilization. Front Cell Infect Microbiol 2021;10:620529.
8. Fraczek M, Kurpisz M. Mechanisms of the harmful effects of bacterial semen infection on ejaculated human spermatozoa: potential inflammatory markers in semen. Folia Histochem Cytobiol 2015;53:201-17.
9. Allow AK, Abdulmogny ASS, Maryam B, Alaw BA. Sperm agglutination, sperm shaky head movement and sperm-cervical interaction tests could be enough for diagnosis of immunological infertility? J Gynecol Womens Health 2017;3:555603.
10. Volz Y, Ebner B, Pfitzinger P, Berg E, Lellig E, Marcon J, et al. Asymptomatic bacteriospermia and infertility-what is the connection? Infection 2022;50:1499-505.
11. Gimenes F, Souza RP, Bento JC, Teixeira JJ, Maria-Engler SS, Bonini MG, et al. Male infertility: a public health issue caused by sexually transmitted pathogens. Nat Rev Urol 2014;11:672-87.
12. Fraczek M, Hryhorowicz M, Gill K, Zarzycka M, Gaczarzewicz D, Jedrzejczak P, et al. The effect of bacteriospermia and leukocytospermia on conventional and nonconventional semen parameters in healthy young normozoospermic males. J Reprod Immunol 2016;118:18-27.
13. Wogatzky J, Wirleitner B, Stecher A, Vanderzwalmen P, Neyer A, Spitzer D, et al. The combination matters--distinct impact of lifestyle factors on sperm quality: a study on semen analysis of 1683 patients according to MSOME criteria. Reprod Biol Endocrinol 2012;10:115.
14. Pluddemann A. Can drinking more water prevent urinary tract infections? The evidence says yes. BMJ Evid Based Med 2019;24:191-2.
15. Eini F, Kutenaei MA, Zareei F, Dastjerdi ZS, Shirzeyli MH, Salehi E. Effect of bacterial infection on sperm quality and DNA fragmentation in subfertile men with Leukocytospermia. BMC Mol Cell Biol 2021;22:42.
16. Domes T, Lo KC, Grober ED, Mullen JB, Mazzulli T, Jarvi K. The incidence and effect of bacteriospermia and elevated seminal leukocytes on semen parameters. Fertil Steril 2012;97:1050-5.
17. Machado A, Cerca N. Influence of biofilm formation by Gardnerella vaginalis and other anaerobes on bacterial vaginosis. J Infect Dis 2015;212:1856-61.
18. Farahani L, Tharakan T, Yap T, Ramsay JW, Jayasena CN, Minhas S. The semen microbiome and its impact on sperm function and male fertility: a systematic review and meta-analysis. Andrology 2021;9:115-44.
19. Bai S, Li Y, Hu MH, Wu L, Shui LJ, Wang XH, et al. Association of sexually transmitted infection with semen quality in men from couples with primary and secondary infertility. Asian J Androl 2022;24:317-22.
20. Toren P, Margel D, Kulkarni G, Finelli A, Zlotta A, Fleshner N. Effect of dutasteride on clinical progression of benign prostatic hyperplasia in asymptomatic men with enlarged prostate: a post hoc analysis of the REDUCE study. BMJ 2013;346:f2109.
21. Said MA, Mehta A. The impact of 5 α-reductase inhibitor use for male pattern hair loss on men's health. Curr Urol Rep 2018;19:65.
22. La Vignera S, Condorelli RA, Balercia G, Vicari E, Calogero AE. Does alcohol have any effect on male reproductive function? A review of literature. Asian J Androl 2013;15:221-5.