Impact of Bacterial Infections on Semen Parameters in Sub-Fertile Men

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Abstract

The male reproductive system is a complex structure crucial for sperm generation, transportation, and release. This study aimed to investigate the correlation between bacterial infections and male fertility by evaluating sperm parameters. A prospective observational study was conducted on 100 sub-fertile men and 30 fertile controls in western Iraq from October 2022 to October 2023. Semen samples were collected and analyzed for bacterial infections and sperm parameters. Results showed prevalent bacteria in seminal fluid, including E. coli, Enterococcus faecalis, and Streptococcus agalactiae. No significant differences in age (p = 0.813) or BMI (p = 0.609) were seen between the control group and the sub-fertile males group. Compared to fertile and sub-fertile males, bacteriospermia altered sperm concentration, motility, and morphology, affecting male fertility. Bacterial infections correlated with decreased semen volume, sperm count, motility, and morphology. Healthy fertile men exhibited superior sperm parameters compared to sub-fertile groups, emphasizing the clinical significance of bacteriospermia on male fertility. This study provides insights into the relationship between bacterial infections and male reproductive outcomes, informing potential therapeutic interventions for male sub-fertility.

Significance | This study demonstrated a correlation between bacterial infections and semen parameters, providing insights for precise therapy strategies in male sub-fertility.

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Introduction

The male reproductive system is an intricate anatomical structure that plays a vital role in the generation, transportation, and release of sperm. The male reproductive system comprises several essential components, namely the testes, epididymis, vas deferens, and a range of auxiliary glands(Obukohwo, Kingsley, Rume, & Victor, 2021) . The process of sperm creation predominantly takes place in the testes, specifically within the seminiferous tubules. Specialized cells undergoes spermatogenesis within those tubules, this result in production of fully mature sperm. After that, the spermatozoa acquire motility from the epididymis, it gain the capability of fertilization. Subsequently, the vas deferens emerges from the epididymis, support the movement of spermatozoa through ejaculation (Mateus et al., 2023; Suede, Malik, & Sapra, 2020). Seminal vesicles and prostate gland which are known as accessory glands, play vital role in production process. These glands produce seminal fluids which enhance the movement of sperm through the female reproductive canal and facilitating fertilization (Tamessar et al., 2021).

Sperm characteristics evaluation is important in male fertility assessment. Sperm characteristics include three essential parameters: sperm count, sperm motility, and morphology. Male reproductive potential is correlated with these three parameters (Oehninger & Ombelet, 2019). Sperm count, also known as sperm concentration, which is the total count of sperm in a specific volume of ejaculate and it is always about 15 million sperm per milliliter. Success of Fertilization process may be limited due to adverse effect

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of oligospermis which mean deviation from standards (Alahmar, Calogero, Sengupta, & Dutta, 2021; Mohammadi et al., 2021).

Sperm motility is the ability of sperms to move forward continuously. Fertility assessment require that 40% or more of the sperm of an ejaculate have the ability to move vigorously(Gacem et al., 2023). Morphology of sperm is the shape and structure assessment of sperm. Standards stipulate that a minimum of 4% of sperm should display a normal form(Lincoln Bastos Farias et al., 2023) . Abnormal sperm morphology which is known as teratospermia, may cause fertility issue as it restrict the sperm capability to move and penetrate the ova (Zhao et al., 2023).

Health of male reproductive system are affected by the bacterial infections. Many diseases like epididymitis and orchitis are sexually transmitted infections including chlamydia and gonorrhea. (Bonner, Sheele, Cantillo-Campos, & Elkins, 2021; Guiton & Drevet, 2023). Infections of the reproductive system may be result from Urinary tract infections (UTIs) (Beltran & Berney, 2020). Moreover , bacteria may cause prostatitis, which is prostate gland infection and these infections can disturb the reproductive organs ability to normally operates resulting in low sperm count, motility, and morphology(Henkel, Offor, & Fisher, 2021; Ivanov, Pavlova, & Atanassova, 2023). Inflammation create an unfavorable environment and as a result, sperm production is impeded (Mändar et al., 2017).

Likewise, in response to infections, the immune system produces reactive oxygen species (ROS),which causes oxidative stress that has adverse effects on sperm DNA quality and morphology. Moreover, inflammatory cytokines that result from the infections may contribute to sperm defects and worsen function (Darbandi et al., 2018; F. Wang, Chen, & Han, 2019).

The rationale to conduct a study in our particular population is to fully investigate the long-term impacts of bacterial infections on sperm parameters. The research aims of this study are focused on investigating the correlation between bacterial infections and male fertility. The hypotheses put forth suggest that bacterial infections have an adverse effect on various aspects of sperm quality, including count, motility, and morphology, hence affecting reproductive health.

Materials and Methods

It is a prospective observational study done at Ar-Razzi IVF hospital in western Iraq. This investigation composed of 100 sub-fertile men and control group consisting of 30 fertile males and they were considered fertile as they achieved natural pregnancy in their first year of marriage. This investigation was conducted over the period spanning from October 2022 to October 2023. The study aimed to examine the impact of bacterial infections on sperm parameters among males with subfertility.

This study's inclusion criteria precisely identify infertile men eligible for participation. . Infertility is indicated by absence of pregnancy after 12 months of marriage due to male factor with normal female factor. Exclusion criteria include men whose spouses have abnormal female reproductive factors, males who have surgery in the past month. Participants who received antibiotics in the past month are excluded. All participants provided informed consent, and the study was conducted following the principles of the Declaration of Helsinki. The confidentiality and privacy of all participants were strictly maintained throughout the research process.

Semen collection and preparation:

It was done by guidelines prescribed by the World Health Organization (WHO) 2022 for the collecting of semen. Semen samples were taken at the lab after 2-5 days of sexual abstinence through masturbation. Semen was collected in a sterile container for analysis (C. Wang, Mbizvo, Festin, Björndahl, & Toskin, 2022).The collected ejaculates underwent a full andrological analysis following a 30-minute process of semen liquefaction at a temperature of 37°C.

The present study involved the evaluation of leukocyte count and semen culture as diagnostic tools for the identification of bacterial infections. Semen parameters comprised the assessment of semen volume, sperm concentration, motility, and morphology.

Culturing of semen and micro and macroscopic analysis:

The process of culturing semen was carried out using Blood Agar Medium and MacConkey Agar Medium. Traditional methods were used to determine the appropriate incubation period and bacterial growth, and the Vitek2 compact was used to diagnose the colonies after they were obtained. The macroscopic analysis encompassed the quantification of semen volume, evaluation of its characteristics. Under typical circumstances, the average volume of semen is generally 1.5 mL or greater, with an expected visual characteristic of being opaque and exhibiting a white-grey coloration. The acceptable pH range is often defined as 7.2 to 8.0, while the process of liquefaction is expected to transpire within a time frame of 15 to 30 minutes (Chamkori, Shariati, Moshtaghi, & Farzadinia, 2016; Chillon et al., 2023).

The evaluation of sperm concentration was conducted utilizing a counting chamber placed beneath a microscope, where the benchmark for a standard concentration was set at 15 million sperm per milliliter or higher. The lowest threshold for motility, which is an indicator of sperm movement, was determined to be 40% of sperm exhibiting motility. Finally, in terms of morphology, the evaluation of the proportion of sperm displaying a normal shape was deemed to be the norm when 4% or greater of the sperm population (Agarwal et al., 2022; Auger, Jouannet, & Eustache, 2016).

The statistical analysis was performed using SPSS version 25.0 to compute important metrics, such as the mean, median and standard deviation, for evaluating the data in the current study.

Furthermore, p-values have been calculated in order to assess the significance of the correlations observed in the study.

Results

Age and Body Mass Index (BMI) distribution:

The range of age for the sub-fertile group was 20-65 years, with a mean of 33.6 ± 7.5 and median 24.50. The BMI range for sub-fertile men was 19.8 to 43.1, with a mean of 26.9 ± 4.9 and median23.42. A p-value of 0.813 and 0.609 showed no statistically significant age and BMI difference between the control and sub-fertile groups, table (1).

Distribution of infertility type in sub-fertile men:

Primary infertility was more prevalent than secondary infertility, with 62% of men experiencing primary infertility compared to only 28% experiencing secondary infertility. Most individuals did not encounter secondary infertility. 72% of individuals did not experience any difficulties in conceiving following a previous successful pregnancy, whereas only 28% encountered challenges with secondary infertility, table (2), figure (2).

Prevalence of different bacterial species in seminal fluid:

The dominant microorganisms identified in the seminal fluid microbial composition are as follows: from 100 sample, culturing was performed and the results showed that : E. coli is found in 13 sample (13%(, Enterococcus faecalis 9 (9%), Streptococcus agalactiae 8 (8%), Staphylococcus epidermidis 6 (6%), Streptococcus Pseudinterunedins 6 (6%), and Mycoplasma hominis 6 (6%). The microorganisms Raouella ornithinolytica, Chlamydia trachomatis, and Candida Spp. were observed in 4 instances each (4%).

The presence of Staphylococcus aureus, Staphylococcus saprophyticus, and unidentified bacteria was detected in 5(5)%, 5 (5%), and 3(3)% of instances, respectively.2 (2%) of cases demonstrate the presence of Mycoplasma genitalium, but no growth is observed in 24 sample (24 %), figure (3).

Semen and sperm parameters correlation with infection of bacterial spp. :

A benchmark for normal semen characteristics is the 30-case control group. Individuals infected with Staphylococcus aureus and Staphylococcu saprophyticus have altered semen characteristics, such as reduced volume (3.8±1.0 ml), total sperm count (224.5±62.4 million and 174.5±111.3 million), total motility (46.0±10.0% and 45.0±7.9%), and sperm concentration (58.0±13.4% and 51.6±12.9%, respectively).

Semen parameters are affected by Streptococcus agalactiae infection, including decreased volume (2.3±1.5 ml), total sperm count (185.7±69.3 million), total motility (39.1±9.4%), and sperm concentration (75.4±11.8%). Both Raouella ornithinolybica and E. coli infections cause a considerable decrease in overall motility (1.5±0.7% and 36.5±7.0%, respectively), suggesting potential deleterious consequences on sperm functioning. Mycoplasma hominis and Mycoplasma genitalium infections affect semen parameters differently. Mycoplasma hominis infection decreases total motility (48.3±11.2%), while Mycoplasma genitalium infection affects sperm concentration (60.0±12.0%) and total motility (65.0 ± 50%)

Undefined microorganisms lead to decreased motility (51.667±14.43%) and sperm concentration (23.33±12.58%). Chlamydia trachomatis infection is linked to decreased volume (2.2±0.9 ml), sperm count (86.25±8.0 million), and motility (22.5±9.4%). In cases of growth absence, the volume (2.54±1.389 ml) matches the control, but the total sperm count (187.5±125.07) is lower. Total motility (36.35±23.78%) and sperm concentration (44.3±29.855%) similarly fall below the control group, table (3).

Semen and sperm parameters of healthy fertile, bacteriospermic, and non-bacteriospermic sub-fertile men:

Table 4 compares semen and sperm parameters in healthy fertile, non-bacteriospermic, and bacteriospermic sub-fertile men. Age differences across groups were not statistically significant. Semen characteristics showed that healthy fertile men had 3.3 ± 0.6 ml and non-bacteriospermic sub-fertile men had mean volume of 2.54±1.389.

Bacteriospermic sub-fertile men had a significantly lower volume of 2.16 ± 1.1 ml (P value = 0.021*). Sperm concentration and total count were considerably greater in healthy men (83.5 ± 3.6 million/ml and 281.6 ± 17.3 , respectively) compared to sub-fertile groups (P values < 0.001* and < 0.0001****). Motility and morphology were significantly better in healthy fertile men (67.3 ± 1.8 and 96.2 ± 1.3, respectively) compared to bacteriospermic (35.0 ± 4.7% and 0.95 ± 1.1) and non- bacteriospermic sub-fertile (36.35±23.78 and 1.19 ± 1.1, P values < 0.0001****).

Discussion

Male sub-fertility is a noteworthy although sometimes disregarded component of reproductive well-being. This study investigates the impact of age, BMI, and microbial of seminal fluid on semen characteristics in men with reduced fertility.

The current study determined that the mean age of men with sub-fertility was 33.6 ± 7.5, and their average BMI was 26.9 ± 4.9. The results of our research are consistent with a previous study conducted in 2016, which found that a significant majority (78.82%) of men who were being evaluated for infertility were over the age of 30(Vilvanathan et al., 2016). In addition, another study found that the age range of participants in both the experimental group and control group was primarily between 30-34 years, with an average BMI of 29.6.

In this specific study, it is important to note that there were no statistically significant variations in age and BMI between the case and control groups composition(Lundy et al., 2021). The

similarities in demographic parameters seen in several research highlight the consistency and significance of our findings within the wider context of evaluating male infertility.

Table 1. Distribution of Age and BMI among control and sub-fertile men

		Control	Sub-fertile men
Age	Range	23 - 44	20 – 65
	Mean± SD	33.3 ± 5.5	33.6 ± 7.5
	Median	23.20	24.50
	P value	0.813	
BMI	Range	18.5 – 39.8	19.8 – 43.1
	Mean± SD	27.4 ± 4.7	26.9± 4.9
	Median	22.83	23.42
	P value	0.609	

Table 2. Infertility type prevalence

	Primary infertility		Secondary infertility	
	No.	%	No.	%
Yes	62	62%	28	28%
No	38	38%	72	72%

Table 3. Semen and sperm parameters regrading infection of bacterial spp.

	No	Volume (ml)	Total count (%)	Total motility (%)	Concentration (%)
Control	30	3.3±0.6	281.6±95.0	67.3±10.1	83.6±20.0
Staphylococcus epidermidis	6	2.5±0.	154.7±45.5	54.1±16.8	61.6±19.8
Staphylococcus aureus	5	3.8±1.0	224.5±62.4	46.0±10.0	58.0±13.4
Staphylococcus saprophyticus	5	3.4±1.5	174.5±111.3	45.0±7.9	51.6±12.9
Staphylococcus hominis	3	1.7±1.0	146.7±108.6	45.0±19.6	73.3±10.0
Streptococcus Pseudinterunedins	6	2.3±0.7	153.5±29.3	48.3±9.8	66.7±11.3
Streptococcu agalactiae	8	2.3±1.5	185.7±69.3	39.1±9.4	75.4±11.8
Raouella ornithinolybica	2	3.5±0.7	44.0±16.0	1.5±0.7	13.5±6.5
E.coli	13	2.9±1.1	186.3±34.3	36.5±7.0	55.9±7.9
Enterococcus faecalis	9	2.9±1.1	111.6±22.7	36.5±7.0	45.1±8.3
Mycoplasma hominis	6	3.0±1.2	195.4±29.5	48.3±11.2	70.0±25.9
Mycoplasm agenitalinm	2	2.7±0.35	180.0±30.0	60.0±12.0	65.0±5.0
Undefined microorganism	3	3.83±1.04	188.3±18.92	51.67±14.43	23.33 ±12.58
No growth	24	2.54±1.38	187.5±125.07	36.35±23.78	44.3±29.855
Chlamydia trachomatis	4	2.2±0.9	86.25±8.0	22.5±9.4	66.2±23.0
Candida Spp.	4	2.8±0.7	150.4±63.19	37.5±10.5	51.2±17.3

Table 4. Comparison of semen and sperm parameters between the healthy fertile men, bacteriospermic and non-bacteriospermic sub-fertile men.

Parameters	Control	Non-Bacteriospermic Sub-fertile men	Bacteriospermic sub-fertile men
No.	30	24	76
Age ***P value***	33.3±5.5	32.5±7.2 N.S	34.3±7.9 N.S
Volume ***P value***	3.3±0.6	2.54±1.389 0.001*	2.16±1.1 0.001*
Concentration ***P value***	83.6± 20.0	44.3±29.855 <0.0001****	59.1±4.0 0.001*
Total sperm count ***P value***	281.6±95.0	187.5±125.077 <0.0001****	161.4±11.9 <0.0001****
Motility ***P value***	67.3±10.1	36.35±23.78 <0.0001****	35.0±4.7 <0.0001****
Normal morphology ***P value***	6.35±0.25	1.19±1.1 <0.0001	0.95±1.1 <0.0001*

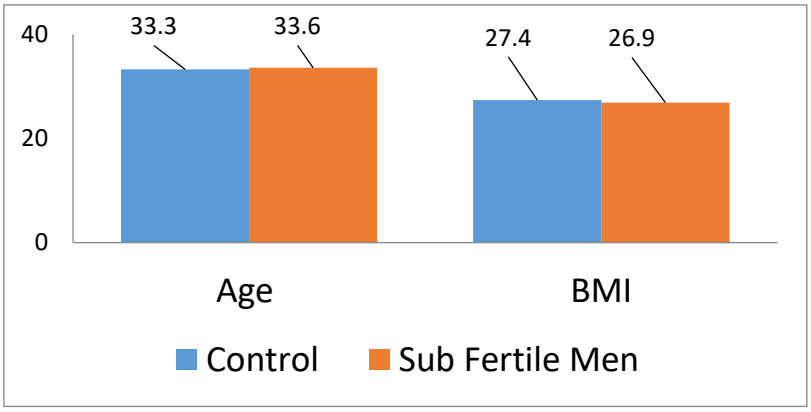

Figure 1. Age and BMI in the studied groups

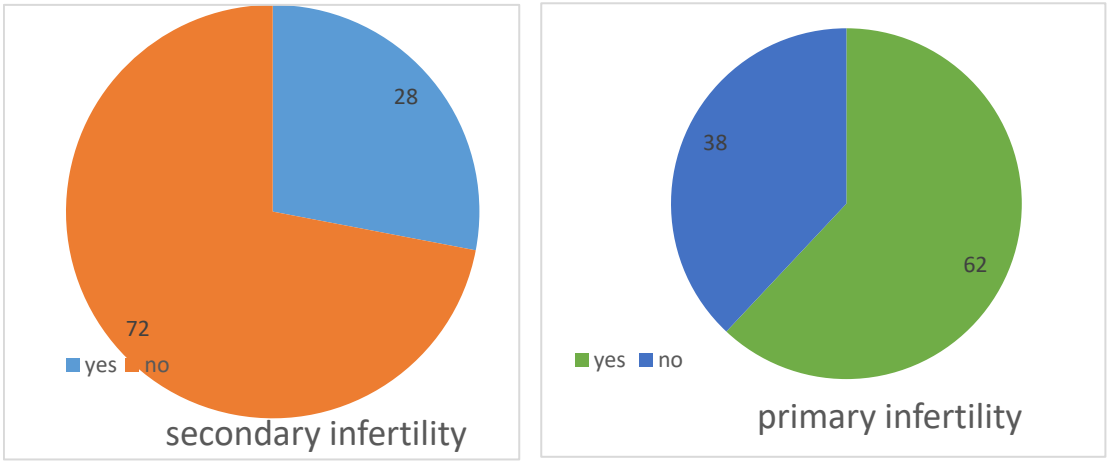

Figure 2. Type of infertility prevalence among sub-fertile men.

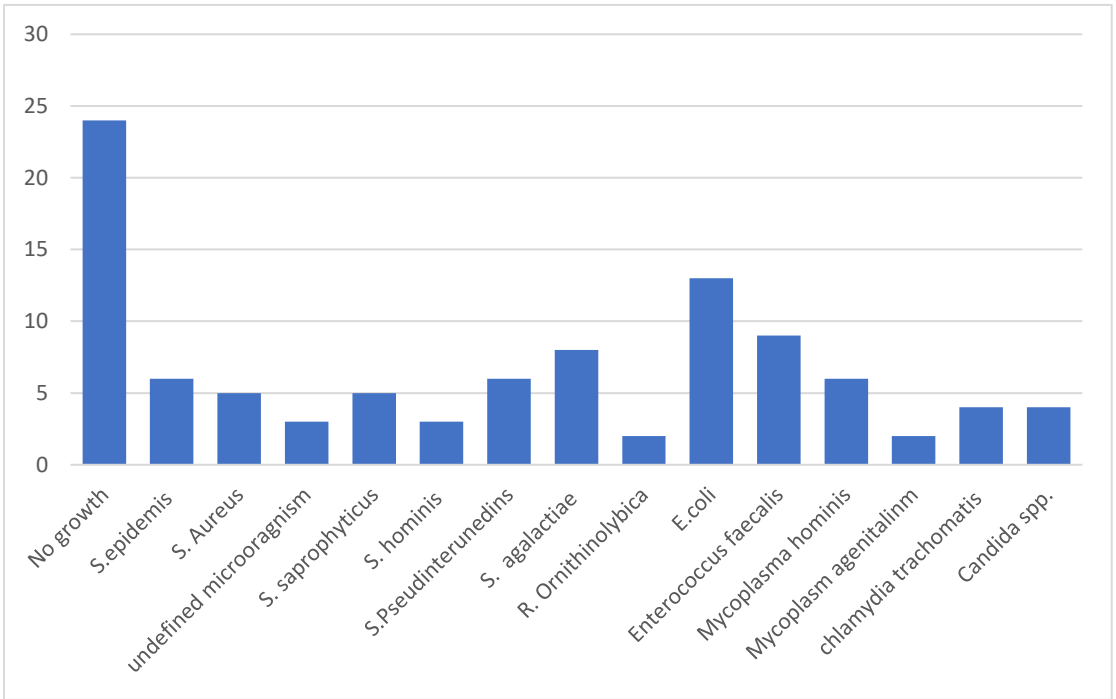

Figure 3. Prevalence of different bacterial spp. in sub fertile men.

Our study revealed a clear trend in the forms of infertility reported in men. Specifically, 62% of the male population experienced primary infertility, whereas 28% presented with secondary infertility. This is consistent with the findings of a prior investigation which showed a 78% occurrence rate for primary infertility and a 22% occurrence rate for secondary infertility (Martins, Panner Selvam, Agarwal, Alves, & Baskaran, 2020). In Nigeria, a recent study conducted in 2023 revealed that 55.17% of cases were classified as primary infertility, while 44.83% were categorized as secondary infertility(Emokpae, Obialor, & Emokpae, 2023). In addition, a study carried out in Gabon demonstrated a different situation, indicating a significant occurrence of secondary infertility at a rate of 66.9%, while only 33.1% had primary infertility (Opheelia et al., 2023).

The present investigation revealed the prevailing microorganisms in seminal fluid, including E. coli (13), Enterococcus faecalis (9), Streptococcus agalactiae (8), Staphylococcus epidermidis (6), Streptococcus Pseudinterunedins (6), and Mycoplasma hominis (6).

The most common bacteria found in Iraq were Enterococcus (32%), followed by Klebsiella spp. (24%), Proteus spp., and Staphylococcus spp. (18% each), and E. coli (16%)(Al-Saadi & Abd, 2019). A recent study conducted in Egypt revealed that Staphylococcus aureus was the most often seen bacterium, accounting for 67.6% of the isolates(Shash, Mohamed, Shebl, Shokr, & Soliman, 2023). This was followed by E. coli at 14.7%, Klebsiella spp. at 11.8%, and Enterococcus spp. at 5.9%. Ghana documented a wide range of bacterial species, including E. coli, S. aureus, U. urealyticum, and Chlamydia trachomatis (Agyepong & Bedu-Addo, 2019). The current study findings support previous observations, highlighting the continuous occurrence of E. coli and Enterococcus faecalis as potentially harmful bacteria in semen samples(Fraczek et al., 2016).

In current investigation, alterations in semen and sperm parameters were observed within the patient group, diverging from some previous research that reported no significant impact on semen parameters in cases of bacteriospermia (McGowan, Burger, Baker, De Kretser, & Kovacs, 1981). However others reported that patients with and without bacteriospermia had a comparable semen and sperm characteristics (Shash et al., 2023).

Notably, in Iraq a study found that the lowest seminal volumes were associated with the presence of E. coli (1.83 ± 0.93) and Klebsiella spp. (2.35 ± 0.82) (Al-Saadi & Abd, 2019), a trend consistent with a study in Iran that linked Mycoplasma hominis and Staphylococcus hominis to reduced progressive sperm motility and semen volume (Pebdeni et al., 2022).

In the Egyptian context, it was shown that patients with bacteriospermia displayed reduced progressive motility, non-progressive motility, and total motility (Shash et al., 2023). This supports the conclusions of another study conducted in 2021, highlighting the persistent detrimental effect of bacterial presence on the ability of sperm to move(Eini et al., 2021).

Similarly, a study conducted in Iraq found that there was a decrease in sperm count when Klebsiella spp. and Enterococcus spp. were present. On the other hand, larger concentrations were observed when E. coli, Staphylococcus, and Proteus were present, compared to the negative control group(Al-Saadi & Abd, 2019).

According to a significant study conducted in 2009, it was shown that infection caused by Enterococcus faecalis is linked to a decrease in sperm concentration. This information adds further historical background to our own research findings(Moretti et al., 2009).

Accordingly, a recent study conducted in Nepal revealed that semen with bacteria had lower levels of sperm concentration, total sperm motility, and normal sperm shape compared to semen without bacteria (Shrestha, Joshi, Vaidya, Shrestha, & Singh, 2023). Moreover, a study conducted in Al-Anbar region, Iraq, revealed a detrimental correlation between different strains of bacteria found in seminal fluid of the male reproductive system and the shape of sperm, emphasizing the extensive influence of bacterial infections on the quality of sperm (Al-Janabi, Jubair, Pemmaraju, Pruthi, & Pruthi, 2014).

In line with these findings, a study in 2018 demonstrated a marked decrease in the overall sperm count (33.78 ± 35.107) among the infected group as compared to the control subjects (69.25 ± 48.276) (Zeyad, Hamad, & Hammadeh, 2018).

This study, supported by evidence from several places, highlights the harmful effect of bacteriospermia on sperm parameters. Bacterial infections consistently correlate with male infertility through the presence of reduced motility, impaired concentration, and modified morphology.

Conclusion

This investigation clarifies some crucial features of male sub-fertility. Although there were no significant variations in age and BMI between the control and sub-fertile groups, the prevalence of primary infertility exceeds that of secondary infertility. The rich microbial composition found in seminal fluid, particularly characterized by specific bacteria, highlights the delicate nature of male reproductive health. The study we conducted demonstrates a connection between bacterial infection and changes in semen parameters, highlighting the negative effect on fertility. Moreover, the contrast between the healthy fertile and sub-fertile groups emphasizes significant differences, emphasizing the possible clinical consequences of bacteriospermia. These findings provide vital insights into the complex relationship between age, bacterial

infections, and male reproductive outcomes, which can help guide more precise therapy strategies for male sub-fertility.

Author contributions

M.M.F., S.Q.T.A.Q., Z.H.A. conducted the experiments, performed the statistical analysis, wrote, edited, and reviewed the article.

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Competing financial interests

The authors have no conflict of interest.

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