West African Journal of Allied Health Sciences

Advancing Allied Health Research in West Africa

www.wajahs.org

eISSN: 3115-4581 pISSN: 3115-4573

Original Article Volume 2, Issue 2, August 2026

Bacteriological, Molecular, and Biochemical Evaluation of Seminal Fluid from Men with Primary Infertility Attending Federal Medical Centre, Bida, Niger State

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Abstract

Introduction: Male infertility is a multifactorial condition in which microbial infections of the seminal fluid play a significant role. Both cultivable and fastidious organisms have been implicated in altering semen parameters, thereby compromising fertility potential. This study aimed to investigate the microbial profile, molecular detection of seminal pathogens, and their association with seminal fluid parameters and biomarkers among men with primary infertility attending the Federal Medical Centre (FMC) Bida, Niger State.

Methods: A total of 207 semen samples from men with primary infertility were analyzed using standard bacteriological techniques and multiplex real-time PCR for microbial detection. Seminal biomarkers (fructose and zinc) were quantified and correlated with semen quality. Data were analyzed using descriptive statistics, Chi-square, and correlation coefficients, with significance set at $p < 0.05$.

Results: *Staphylococcus aureus* (40.1%) and *Escherichia coli* (17.4%) were the predominant cultivable isolates, while molecular assays detected *Ureaplasma parvum* (9.7%), *Ureaplasma urealyticum* (9.2%), *Gardnerella vaginalis* (7.7%), *Chlamydia trachomatis* (7.2%), and *Mycoplasma genitalium* (6.8%) as major fastidious organisms. Abnormal semen parameters were significantly associated with microbial infections, including asthenozoospermia (54.1%, $p = 0.005$), teratozoospermia (54.6%, $p = 0.043$), and azoospermia (23.2%, $p = 0.055$). Elevated leukocyte counts were also observed (46.4%, $p = 0.023$). Biomarker analysis revealed elevated fructose in oligospermic and asthenozoospermic men ($r = 0.994$, $p = 0.002$; $r = -0.733$, $p = 0.001$), while zinc deficiency correlated significantly with reduced sperm motility and concentration ($r = 0.817$, $p = 0.001$).

Conclusion: Seminal fluid infections, particularly by both cultivable and molecularly detected pathogens, significantly impair semen quality and are major contributors to primary infertility.

Keywords: male infertility; seminal fluid; bacteriospermia; multiplex real-time PCR; *Ureaplasma*; *Chlamydia trachomatis*; leukocytospermia

 DOI: 10.67891/yrmq1r39

Received: May 1, 2026 Revised: June 10, 2026 Accepted: June 15, 2026

1.0 Introduction

Infertility is usually defined as the inability of a couple to conceive after 1 year of unprotected, frequent sexual intercourse. It affects about 15% of all couples in the United States and at least 180 million couples worldwide. Male infertility is defined by the World Health Organization (WHO) as the inability of a male to make a fertile female pregnant for a minimum of 1 year of regular unprotected intercourse (World Health Organization, 2025). In Africa, infertility is a major concern mostly because of the negative social impact such as iso-

lation, disinheritance, stigmatization and even divorce in some cases (Nsabimana et al., 2024). In Sub-Saharan Africa (SSA), infertility poses a significant challenge, with prevalence rates reaching as high as 30% in some populations (Gedef et al., 2025). The primary underlying cause of high infertility rates in SSA is attributed to infections, particularly sexually transmitted infections (STIs), advanced maternal age, malnutrition, and inadequate access to healthcare, lack of awareness regarding reproductive health and limited access to fertility treatments (Mabweazara, 2025).

In the past, Africans held the ancient socio-cultural beliefs that women are to be blamed for infertility issues among couples thereby effacing the fact that men are also in need of reproductive health care (Dierickx et al., 2021). This assumption also contradicts the observations that couple infertility is solely due to a male factor in about 20% of the cases and contributes to the couple infertility in another 30–40% (Shade Iwelumor et al., 2022). Agarwal et al. (2021) reported 2.5%–4.8% male factor infertility in Sub-Saharan Africa. In Nigeria, it is reported that the male factor is responsible for 40–50% of all infertility (Akinwalere and Ezeike, 2025). Bacterial colonization of male reproductive tissues and fluids, and its impact on sperm quality and function, has gained attention recently (Tvrdá et al., 2022). Infections, a key cause of urogenital inflammation, can hinder assisted reproductive technology (ART) success (Eini et al., 2021).

Testicular, epididymal, and prostatic injuries from infections can impair spermatogenesis (Eini et al., 2021). This study aimed to (i) determine the prevalence of cultivable and fastidious microorganisms in seminal fluid using standard bacteriological techniques and multiplex real-time PCR; (ii) assess the association between microbial infection and semen parameters; and (iii) evaluate seminal biochemical biomarkers among infertile men attending FMC Bida, Nigeria.

Materials and Methods

Study Area

The study was conducted among male patients with primary infertility that attended Federal Medical Center Bida, Niger State.

Study Design

A descriptive cross-sectional research design was adopted for this study. The study describes the prevalence of microbial infections and the status of certain molecular markers among the participant and also investigates the association between microbial organisms and primary infertility respectively.

Study Population

A total of 207 semen samples were collected from consented male patients with primary infertility that attended Federal Medical Center Bida, Niger State.

Inclusion and Exclusion Criteria

All 207 semen samples from men undergoing primary infertility investigations at Federal Medical Centre Bida, were included. While samples of individuals with a history of gonadal pathologies, genital surgeries, individuals on antibiotics therapy and seminal fluid collected after 7 days of sexual abstinence were excluded.

Ethical Consideration

Ethical approval for the research was obtained from Health Research Ethics and Committee of Federal Medical Centre, Bida, Niger State respectively. Informed consent was obtained from the patients prior to sample collection (FMCB/HCS/HREC/APPR/VOL2/39/21).

Instruments of Data Collection

The instruments used for data collection in this study include: interviewer administered questionnaire, laboratory sample bottles, and informed consent form.

Interviewer Administered Questionnaire

The interviewer administered questionnaire was used to obtain the following data: participant's unique identifier number, socio-demographic data and risk factor associated data.

Laboratory Sample Bottles

Sterile universal sample bottles were used to collect 5ml freshly ejaculated sperm from the study participants, labelled with the participants' unique ID and time of sample collection.

Informed Consent Form

Written informed consent was obtained from each participant by voluntarily signing the inform consent form after due orientation and enlightenment on the objectives and the significant of the research prior to sample collection.

Pre-Testing of the Questionnaire

Eighteen questionnaires were used to pre-test the validity of the questionnaire before embarking on the research proper.

Data Collection Method

Data collection was by interview conducted with the aid of research assistants who had no conflicts of interest in the research work.

Sample Size Determination

Sample size for the study was determined using 14.3% average prevalence from study (Garba-Alkali et al., 2018). Sample size was estimated using the Kish (1965) formula for cross-sectional studies:

$$n = \frac{Z^2 pq}{d^2}$$

Where n is the estimated sample size; Z is the standard normal deviation usually set at 1.96, which corresponds to the 95% confidence interval; p is the prevalence of semen profile of male partner derived from previous studies, which is 14.3% (0.143) (Garba-Alkali et al., 2018); q is the complementary proportion equivalent to one minus p $(1 - p)$, that is, $1 - 0.143 = 0.857$; and d is the degree of absolute precision. A relative precision of 5% (0.05) was used to achieve n in estimate of semen profile of male partners at 95%.

$$n = \frac{1.96^2 \times 0.143 \times (1 - 0.143)}{0.05^2} = 188.32$$

This gave a minimum semen sample size of 188, and with an attrition rate of 10%, a final sample size of 207 semen samples was used.

Sample Collection

In this study, 207 semen samples were collected from male patients with primary infertility attending Urology clinic, Gynecology clinic and General Out Patient Unit of Federal Medical Centre, Bida, Niger State. The samples were collected in privacy in medical microbiology laboratory and delivered to the laboratory within one hour after collection into a sterile, clean and wide mouthed, screwed capped container and labelled with the participants' ID, period of abstinence, date and time of collection.

Laboratory Analysis

Semen Analysis

The seminal fluids were examined according to WHO (2025) guidelines for liquefaction time/viscosity, volume (in millilitre), percentage motility, sperm count, leukocyte count and sperm morphology, as follows.

Liquefaction Time and Viscosity

The samples were placed in an incubator and left for 40 minutes to liquefy. After liquefaction, the viscosity of the sample was measured by gently aspirating the sample into a wide-bore (approximately 1.5 mm diameter) plastic disposable pipette, allowing the semen to drop by gravity and observing the length of any thread. A normal sample leaves the pipette in small discrete drops. If viscosity is abnormal, the drop will form a thread more than 2 cm long.

Volume

Normal semen liquefies within 60 minutes after which the volume was measured using a small graduating cylinder.

Percentage Motility

One drop of well mixed semen was placed on a slide and covered with a clean cover glass. The specimen was mounted on a microscope using x10 objective to give a good contrast and x40 objective to examine several fields to observe motility. A total of 100 spermatozoa were counted and observed for motility. The percentage motile and nonmotile spermatozoa were recorded.

Sperm Counts

The semen was diluted 1:20 with sodium bicarbonate–formalin diluting fluid. Using a Pasteur pipette, an improved Neubauer counting chamber was filled with well mixed diluted semen and the spermatozoa were allowed to settle for 5 minutes. Using the x10 objective, the number of spermatozoa in an area of $2mm^2$ was counted. To calculate the number of spermatozoa in 1ml of fluid, the number counted was multiplied by 100,000.

Sperm Morphology

Aliquot (5–10μl) of semen was placed 2cm away from the end of the slide. Using a second slide placed at angle 45°, a thin smear was made, allowed to air dry, fixed in 95% methanol for 1 hour and stained using Diff–Quick staining method as recommended by Mazzuchini et al. (2024). The stained slide was examined using x100 oil immersion bright field objective.

Leukocyte Count

Liquefied semen, Phosphate buffered saline (PBS), and working Entz solution (1:1:2) were prepared in a test tube and incubated at room temperature for 5 minutes. An improved Neubauer counting chamber was charged with well mixed diluted mixture and allowed to settle for 5 minutes. Using the x10 objective, the number of peroxidase positive WBC in an area of 2mm^2 was counted. The total number of cells counted was multiplied by four (due to the dilution factor) to determine the concentration of WBCs per millilitre of semen.

Detection and Identification of Microorganisms in Semen Samples (Bacteriospermia) using Standard Bacteriological Method

A 1:10 dilution of the semen sample was made with sterile saline solution and centrifuged at 1500 rpm for 15 minutes. The supernatant was decanted, the sediments resuspended in 100 μl of sterile saline solution with 10% glycerol and centrifuged to pellet the bacterial cell. The cell pellet was seeded quantitatively using a calibrated loop on agar plates (Blood agar, Chocolate agar and MacConkey agar) with isovitalex (1%) and were incubated in 5% CO_2 at 37°C for 24 hours. Bacteria were identified by Gram staining and Bio-Mérieux Api systems (Bio-Mérieux, Marcy l'Etoile, France). Semen culture was considered positive when the number of colonies was $\geq 10^4$ CFU/ml for Gram positive cocci and $\geq 10^5$ CFU/ml for Gram negative organisms (Eini et al., 2021).

Microbial Evaluation of Semen Samples by Multiplex Real-Time Polymerase Chain Reaction (M-PCR)

DNA Extraction Method using Virus DNA/RNA Extraction Kits (Spin Columns) Proteinase K (25 μl) was pipetted into a 1.5 mL microcentrifuge tube, and 200μL of the semen sample was added. Nucleic acid precipitation aid (30μL) and 500μL of Lysis Buffer were added to the tube respectively, mixed by vortexing for 30 seconds, and incubated for 5 min at room temperature. The mixture was pipetted into the spin column placed in a 2 ml collection tube and centrifuged at 12000rpm for 1 minute; the flow-through was discarded. The spin column was washed first with washing buffer 1 (500μL), centrifuged at 12000rpm for 1 min and the flow-through discarded, then the same was repeated using 500μL of washing buffer 2. The spin column was placed back into the collection tube and centrifuged at 12000rpm for 1 min, discarding the flow-through and collection tube respectively. The spin column was placed in a clean 1.5 mL microcentrifuge tube, 50 μL Elution Buffer was added onto the silica-based membrane, and centrifuged at 12000rpm for 1 min to elute. The extracted nucleic acid was stored at −20°C.

Multiplex Real-Time Polymerase Chain Reaction (M-PCR) The kit has an Internal Control (IC), which monitors PCR inhibitors in the sample to be tested by indicating normal or abnormal (IC) results to avoid false negative PCR results. This kit contains 4 bottles of reaction system (master mix), and the specific test items were carried out according to the manufacturer's protocol.

Preparation of Reagents The STD-12 PCR Master Mix 1–4 bottles were reconstituted by adding 960μL of re-dissolved diluent to each lyophilized tube, vortexed for 1 minute, and allowed to stand for approximately 60 seconds until the liquid was clear and transparent.

Preparation of Reaction Mixture 20μL of the template DNA was pipetted into PCR reaction tubes for each of the master mixes, and 5μL of the extracted DNA was added (one sample added to four reaction tubes of the respective master mixes). The tubes were closed gently. 5μL each of positive and negative control were added to the reaction tubes, which were then centrifuged for 15 seconds, avoiding bubbles, and delivered into the nucleic acid amplification region.

PCR Amplification The PCR conditions were set as: initial denaturation at 95°C for 2 minutes (1 cycle), followed by 45 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 30

seconds. Fluorescence was measured during the 60°C step in each cycle to monitor amplification in real time. The amplification detection channels were selected for each organism as well as Positive, Negative controls (FAM/VIC both) and Internal control (CY5). The kit contains 4 reaction systems; the same procedures were repeated following the kit instructions.

Data Statistical Analysis

The data obtained from this study were analyzed using IBM® SPSS. Frequency and percentages for demographic variables were presented using tables and charts for easy explanation. Chi-square (χ^2) was also done to test for association between the research variables. Level of significance was set at $p \leq 0.05$.

Results

Table 1: Evaluation of seminal fructose concentration in normospermic, oligospermic, azoospermic and asthenozoospermic patients

Variable	Fructose: RR [150mg/dl–8.34mmol/l]	Frequency
Normospermia	8.1	50
	8.3	69
	8.11	88
	Corr. Coeff. (r): 0.044; p=0.005	
Oligospermia	12.4	72
	11.4	55
	13.1	80
	Corr. Coeff. (r): 0.994; p=0.002	
Asthenozoospermia	15.3	73
	16.1	63
	14.4	71
	Corr. Coeff. (r): −0.733; p=0.001	
Azoospermia	8.9	82
	9.3	67
	7.6	58
	Corr. Coeff. (r): 0.063; p=0.001	

RR: Reference Range.

Table 2: Evaluation of seminal fluid zinc level of normospermic, oligospermic, azoospermic and teratospermic patients

Variable	Zinc: RR [72–127 μg/dl]	Frequency
Normospermia	6.3	86
	7.1	51
	9.4	70
	Corr. Coeff. (r): −0.200; p=0.003	
Oligospermia	19.8	60
	14.4	68
	18.3	79
	Corr. Coeff. (r): −0.181; p=0.001	
Teratospermia	14.3	81
	12.5	57
	14.5	69
	Corr. Coeff. (r): 0.817; p=0.001	
Azoospermia	22.4	74
	19.9	83
	20.6	50
	Corr. Coeff. (r): −0.018; p=0.008	

RR: Reference Range.

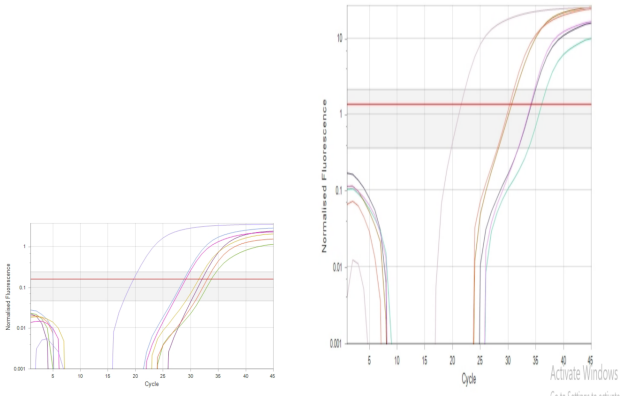

Figure 1: Amplification curve for *Ureaplasma urealyticum* and *Ureaplasma parvum*

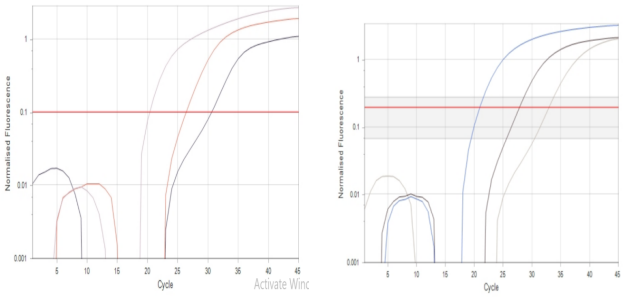

Figure 2: Amplification curve for *Mycoplasma genitalium* and *Mycoplasma hominis*

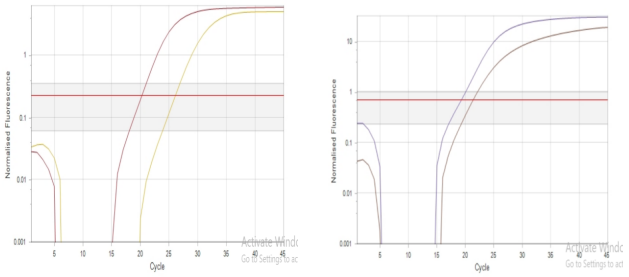

Figure 3: Amplification curve for *Chlamydia trachomatis* and *Candida albicans*

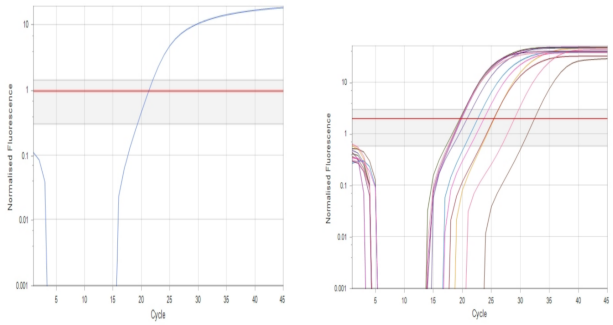

Figure 4: Amplification curve for *Treponema pallidum* and Internal Control (IC)

Table 3: Microbial assessment of seminal fluid using multiplex real-time polymerase chain reaction (M-PCR)

S/N	Microbial Organism	Freq.	Prev. (%)
1	*Ureaplasma parvum*	20	9.7
2	*Ureaplasma urealyticum*	19	9.2
3	*Neisseria gonorrhoeae*	0	0.0
4	*Gardnerella vaginalis*	16	7.7
5	*Chlamydia trachomatis*	15	7.2
6	*Mycoplasma genitalium*	14	6.8
7	Herpes simplex virus 1	0	0.0
8	*Mycoplasma hominis*	5	2.4
9	*Candida albicans*	3	1.5
10	*Treponema pallidum*	0	0.0
11	*Trichomonas vaginalis*	0	0.0
12	Herpes simplex virus 2	0	0.0

Table 4: Effect of microbial infection on seminal fluid parameters from patients with primary infertility attending FMC Bida, Niger State

Semen Characteristics	**Total (N=207)**	**Disorder (%)**	**No. Infected (N=138)**	**No. Non-Infected (N=69)**	p**-Value**
Hypospermia	67	32.4	39	28	0.1035
Asthenozoospermia	112	54.1	87	25	0.0005
Teratozoospermia	113	54.6	68	45	0.0430
Azoospermia	48	23.2	38	10	0.0547
Oligospermia	74	35.7	54	20	0.1999
Leukocytospermia	96	46.4	82	14	0.0000
Hyperviscous	67	32.4	51	16	0.0660

$p < 0.05$ considered statistically significant.

Discussion

A notable finding in this study is the relatively high proportion of culture-negative samples (33.3%), which highlights the limitations of conventional bacteriological techniques. Similar observations have been reported by Eini et al. (2021), who noted that many pathogens implicated in infertility are fastidious or intracellular and therefore not easily detected by routine culture. The application of multiplex real-time PCR in this study revealed additional pathogens such as *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Gardnerella vaginalis*, and *Chlamydia trachomatis*, with an overall positivity rate of 44.4%. This supports findings by Baud et al. (2019) and Pagliuca et al. (2021), who emphasized that molecular diagnostics significantly enhance detection rates of sexually transmitted and fastidious organisms in semen.

A major strength of this study is the clear demonstration of a statistically significant association between infection and key semen abnormalities, particularly asthenozoospermia, teratozoospermia, and leukocytospermia. These findings are in agreement with previous studies (Eini et al., 2021), which reported that infections impair sperm motility and morphology through inflammatory processes and oxidative stress.

The evaluation of seminal biomarkers in this study further strengthens the understanding of infertility pathophysiology. The observed elevated fructose levels in oligospermic and asthenozoospermic men may reflect compensatory secretory activity of the seminal vesicles or altered energy metabolism. Fructose serves as a key energy source for sperm motility, and its dysregulation has been associated with poor semen quality (Agarwal et al., 2021). Conversely, zinc deficiency was significantly associated with reduced sperm motility and concentration, consistent with previous findings that zinc is essential for spermatogenesis, sperm membrane stability, and antioxidant defense (Tvrdá et al., 2022).

6. Limitation

Limitations of this study include: (i) single-centre based and cross-sectional design, (ii) absence of a fertile control group, and (iii) inability to establish causation from observed associations.

5. Conclusion

Microbial infections significantly contribute to male infertility. Infections primarily affect sperm function (motility and morphology) through inflammation and oxidative stress.

Seminal biomarkers such as fructose and zinc are important indicators of reproductive health.

Considering the socio-economic burden posed by infertility on families and society at large, involvement of cultivable and fastidious microbes, early detection by PCR techniques and treatment is recommended.

References

Agarwal, A., Baskaran, S., Parekh, N., Cho, C., Henkel, R., Vij, S., Arafa, M., Panner Selvam, M. K., and Shah, R. (2021). Male infertility. *The Lancet*, 397(10271):319–333.

Akinwalere, F. and Ezeike, A. (2025). Male infertility in Nigeria: Causes, symptoms, diagnosis, and treatment. Datelinehealth Africa Inc.

Baud, D., Pattaroni, C., Vulliemoz, N., Castella, V., Marsland, B. J., and Stojanov, M. (2019). Sperm microbiota and its impact on semen parameters. *Frontiers in Microbiology*, 10:424750.

Dierickx, S., Oruko, K. O., Clarke, E., Ceesay, S., Pacey, A., and Balen, J. (2021). Men and infertility in The Gambia: limited biomedical knowledge and awareness discourage male involvement and exacerbate gender-based impacts of infertility. *PLOS ONE*, 16(11):e0260084.

Eini, F., Kutenaei, M. A., Zareei, F., Dastjerdi, Z. S., Shirzeyli, M. H., and Salehi, E. (2021). Effect of bacterial infection on sperm quality and DNA fragmentation in subfertile men with leukocytospermia. *BMC Molecular and Cell Biology*, 22:42.

Garba-Alkali, A. E., Adesiyun, A. G., and Randawa, A. J. (2018). Semen profile of male partners of women attending infertility clinic in Zaria, Nigeria. *Tropical Journal of Obstetrics and Gynaecology*, 35:256–260.

Gedef, G. M., Taye, E. B., Mohammed, O. Y., Abegaz, M. Y., Asratie, M. H., and Andualem, F. (2025). Prevalence of infertility and its risk factors in Sub-Saharan Africa: a systematic review and meta-

analysis. *Contraception and Reproductive Medicine*, 10(1):73.

Mabweazara, G. M. (2025). Access to assisted reproductive technology: a qualitative study of couples with infertility in Cape Town, South Africa. *Women's Reproductive Health*, 12(1):179–198.

Mazzuchini, M., Novello, G., and Segabinazzi, L. (2024). Modified diff-quick staining for canine sperm morphology. *Clinical Theriogenology*, 16.

Nsabimana, A., Ninihazwe, A., Irambona, H., and Niyonkuru, E. (2024). The impact of infertility: a holistic examination of physical, emotional, and social effects on couples. *IJMR*, 10(6):308–316.

Pagliuca, C., Cariati, F., Bagnulo, F., Scaglione, E., Carotenuto, C., Farina, F., Carraturo, F., Vitiello, M., Strina, I., Alviggi, C., Colicchio, R., Tomaiuolo, R., and Salvatore, P. (2021). Microbiological evaluation and sperm DNA fragmentation in semen samples of patients undergoing fertility investigation. *Genes*, 12(5):654.

Shade Iwelumor, O., Suraya Syed Jamaludin, S., and Seun Babatunde, K. (2022). Social construction of female infertility: a qualitative study of women with female factor infertility diagnosis. *Journal of International Women's Studies*, 24(1):9.

Tvrdá, E., Ďuraka, M., Benko, F., and Lukáč, N. (2022). Bacteriospermia — a formidable player in male subfertility. *Open Life Sciences*, 17(1):1001–1029.

World Health Organization (2025). Guideline for the prevention, diagnosis and treatment of infertility. World Health Organization.