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Original Research Article

Semen parameters and the incidence and effects of bacteriospermia in male partners of infertile couples attending a fertility clinic in the Kumasi Metropolis, Ghana

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ABSTRACT

Background: Inability to conceive after at least 12 months of unprotected regular intercourse has been recognized as a very serious problem for couples especially those who are at the reproductive age and legally married. Bacterial infection of male genitourinary tract is considered as one of the promoting factors for male infertility. These pathogenic bacteria in the ejaculates can induce a defect in semen parameters, such as sperm count, morphology and motility which are predictors of male fertility potential. The study was conducted to assess the semen quality and the incidence of bacteriospermia in male partners of infertile couples attending fertility clinic at Trust Care Specialist Hospital, Kumasi.
Methods: Semen samples of 300 male partners of infertile couples were collected and evaluated by WHO guidelines. The samples were also cultured using standard bacterial culture techniques.
Results: Oligozoospermia was the highest semen abnormality identified. This was followed by Teratozoospermia. Other semen abnormalities identified were azoospermia, asthenozoospermia, oligoteratozoospermia, asthenoteratozoospermia, oligoasthenozoospermia, and oligoasthenoteratozoospermia. 67 (22.3%) out of the 300 samples showed significant bacterial growth. Eight different bacteria species were isolated. *E. coli*, was isolated from 27 of the samples, *S. aureus* from 13 of the samples, U. urealyticum from 10 of the samples, Chlamydia trachomatis from 9 of the samples, Pseudomonas spp from 5 of the samples *Proteus spp*, *Klesbsiella spp* and *M. morganii* were each isolated from one sample.
Conclusions: Although bacteria were isolated in 22.3% of the semen samples, their presence imparted negatively on the semen quality. This suggests that bacterial infection should be one of the investigations to be carried out in the treatment of infertility. Even when count, motility, and morphology look normal other parameters such as infection and sperm DNA should be investigated during the treatment of infertility.

Keywords: Bacteriospermia, Infertility, Semen, Spermatozoa, Normospermia

INTRODUCTION

The burden of infertility has been recognized globally as a very serious problem for couples especially those who are at the reproductive age and legally married. Infertility is usually defined as inability to conceive after at least 12 months or more of unprotected regular intercourse.[1]

Approximately 15% of couples are unable to conceive after one year of unprotected intercourse. About 20% of

infertility in couples is due solely to male factors and contributes to another 30-40%.[2]

The principal common causes of infertility are male factors such as sperm abnormalities, female factors, such as ovulation dysfunction and tubal pathology, combined male and female factors, and idiopathic infertility, where no clear cause could be identified.[3-7] One out of five married couples demonstrates primary infertility. Despite advance in evaluation, idiopathic male infertility (IMI) affects nearly 10-15% of men in their prime reproductive age.[8] Infections could lead to infertility through the following presumed mechanisms; attachment of bacteria to sperm, some bacterial producing an immobilizing factor, recruitment of the immune system and glandular-function alteration.[9] The pathogenic bacteria in the ejaculates can prompt a defect in semen parameters, such as reduced sperm count, poor morphology and motility.[10]

Traditionally, the female is held responsible for the failure to conceive. However, the evaluation of the capacity of the male reproduction is found to be lacking in not less than 50.0% of infertile couples.[11] The fecundity of the male most often depends on the quality of semen produced. Current studies have revealed that the simple appearance of bacteria in semen samples may compromise the quality of sperm.[12] Fertility is usually impaired by infection by different mechanisms, including spermatogenesis damage, impairment of sperm function, and seminal tract obstruction.[13] A study in Ghana among rural population on infertility revealed a prevalence of 11.8% among women and 15.8% among men.[14] This affirms that infertility in males is a genuine problem in many countries whether developed or developing which Ghana happens to be one.

The results of most of the researches about male infertility revealed that, the major cause of male infertility has been seminal fluid abnormality. However, not much studies have been sighted in Ghana and specifically in the Kumasi metropolis on the kind of bacterial pathogen present in semen samples of male partners of infertile couples. This study therefore sought to investigate the kind of bacteria pathogen in semen produced by male partners of infertile couples in the Kumasi metropolis.

METHODS

Study Population and sample

This descriptive cross-sectional study was conducted in the Ashanti Region of Ghana specifically in the Kumasi Metropolis. Three hundred (300) male partners of infertile couples visiting the fertility clinic of Trust Care Specialist Hospital at South Suntreso in the Kumasi Metropolis were used for the study. Participants who agreed to take part in the study were made to sign a consent form. The study had the approval of the Research Ethics Committee of the Trust Care Specialist Hospital and the Committee on Human Research, Publications and Ethics (CHRPE) of Kwame Nkrumah University of Science and Technology. Each participant was registered with a unique identification code that corresponded with the code on the containers for both semen and blood samples.[15]

Semen collection and analysis

Semen was obtained from 300 male partners of infertile couples attending the fertility clinic of Trust Care Specialist Hospital at South Suntreso in the Kumasi Metropolis. Participants were instructed to first pass urine, wash their hands and penis with soap, rinse with water to reduce the risk of contamination of the specimen with commensal organisms from the skin, dry hands and penis with a fresh disposable towel prior to semen collection. Semen collection was by masturbation into a sterile container after 2-7 days of abstention from sexual intercourse.[16,17] Semen samples were incubated at 37°C and analyzed within one hour of collection according to WHO guidelines17 at the MediLab Diagnostic Centre. Semen parameters determined included appearance, volume, pH, viscosity, liquefaction, concentration, motility, morphology, presence of other cells like epithelial cell or round cell, and sperm agglutination.

Appearance

The appearances of the semen samples were determined just after liquefaction. This was carried out initially by inspecting the colour of the samples at room temperature. Alteration in visual appearance of the samples such as colour, clearness and presence of mucous streaks were noted. Semen samples were classified under two main groups: Normal and Abnormal. The abnormally coloured samples were also sub grouped into Blood-stained and Non-blood stained.[17]

Volume

The volume of each semen sample was measured by pouring the sample into a graduated measuring cylinder and volume measured in milliliters.

pH

The pH of each seminal sample was determined using a narrow range pH paper (pH 6.4-8.0) by spreading a drop of the sample evenly onto the pH paper. After 30 seconds, the color of the instilled area was compared with the calibrated strip.

Viscosity

The viscosity of the sample was determined with the aid of Pasteur pipette. A drop of semen was allowed to drop by gravity and the length of the thread was observed carefully and recorded for each sample. Viscosity of the samples were classified as very high, high normal and

low. A normal sample displayed a thread of length about 2cm upon falling from the Pasteur pipette. Threads that were more than 2 cm were classified as slightly high in viscosity. Samples that formed threads that were more than 3 cm were classified as having high viscosity. Samples which showed threads that were less than 2 cm were classified under low viscous semen.[17]

Concentration

The concentration of the sperm was assessed using a Neubauer counting chamber according to the WHO methods.[17]

Total motility

Total motility of the samples was done by applying a drop (10–15 µl) of the well mixed liquefied sample onto a slide, covered with 22×22 cover slip. The sample was focused using the 10× objective. The condenser iris was closed sufficiently to give a good contrast. Several fields were examined using the 40× objective to assess motility under the microscope using x40 objective lens. The microscopic scanning was done systemically and accordingly motility of each Spermatozoon encountered was graded a, b, c and d, that is

a. Rapid progressive motility
b. Slow or sluggish progressive motility,
c. Non-progressive motility
d. Immotile.

The number of spermatozoa in each category was counted with the aid of a laboratory counter. Usually, four to six fields were scanned to classify 100 successive spermatozoa. All motile spermatozoa with the ones that had their heads moving were recorded. Sperm morphology and vitality were done by using pap and eosin-nigrosin staining technique.

Sperm morphology

A smear was prepared for assessment of the morphology for each sample. The slide was well cleaned and then washed in 70% ethanol and air-dried. 5µl of semen was applied to the slides. Another slide faced down, was placed over so that the semen spreads between them. The two slides were gently pulled apart to make two smears simultaneously. These slides were fixed with 95% v/V ethanol for 5 - 10 minutes and allowed to air-dry.17 The smear was washed with sodium bicarbonate-formalin solution to remove any mucus which may be present and rinsed several times with changes of water. The smears were then flooded with diluted (1 in 20) carbol fuchsin, crystal violet solution and allowed to stain for 2 minutes and then the stain was washed off with water. Lugol's solution (mordant) was added to the smear for 1 minute and washed with distilled water. The smear was then counterstained with dilute (1 in 20) Loeffler's methylene blue safranin (0.1%) solution for 2 minutes and washed with distilled water, drained and air-dried. The preparation was examined for normal and abnormal spermatozoa using the ×40 objective. The ×100 objective was used confirm abnormalities of the morphology of the spermatozoa and the other cellular elements in the smear. The slides were then examined systematically from one microscopic field to another and 100 spermatozoa were assessed, and the percentages of normal and abnormal spermatozoa were recorded. The following abnormalities were all grouped under abnormal sperms: Head (greatly increased or decreased in size, abnormal shape and tapering head- pyriform, acrosomal cap absent or abnormally large, nucleus contains vacuoles or chromatin unevenly distributed, two heads, additional residual body); Middle piece (absent or markedly increased in size, appears divided-bifurcated, angled where it meets tail); Tail (absent or markedly reduced in length, double tail, bent or coiled tail).

Culturing

Liquefied semen samples were cultured unto Blood Agar, Chocolate Agar and MacConkey Agar (Lab M Limited, Topley house, 52 Wash lane, Lancashire, UK) at 37°C for 24 hours. 0.1ml of the specimen was inoculated on the Blood, MacConkey, and Chocolate agars. All the media were prepared according to manufacturer's instructions. The Chocolate agar was incubated in anaerobic jar at 37o C while the other media were incubated in aerobic environment at 37o C for 24hours. The cultured plates were examined for bacteria using standard methods. Aseptic techniques were strictly adhered to during processing of each of the specimen and all standard operating procedures were rigorously followed. The bacteria species isolated were identified by gram staining, and other biochemical tests including the coagulase, indole, and catalase tests. Antibiotic resistance evaluations were not carried out in this research.

Bacterial counts less than 1× 103 CFU/mL was considered as negative cultures. Counts from 1×103 CFU /mL to 3×103 CFU/ mL or counts up to 3×103 CFU mL were considered as positive cultures. Per World Health Organization guidelines,11 a bacterial concentration in the ejaculate up to 1×103 bacteria/mL is a significant bacteriospermia.

Gram staining

A colony of the isolates was picked and used for the gram staining to determine the organism's gram reaction. A loopful of tap water was placed on a slide. A sterile cool loop was used to transfer a small sample of the colony to the drop, and emulsified. The film was allowed to air dry. The dried film was fixed by passing it briefly through the Bunsen flame two or three times without exposing the dried film directly to the flame. The slide was flooded with crystal violet solution for 30 to 60 seconds and washed briefly with tap water and drained. Flood slide was then flooded with Gram's Iodine solution and

allowed to act (as a mordant) for about one minute. The tap water was washed off and drained. Excess water was removed from slide and blotted with filter paper, so that alcohol used for decolourization was not diluted. The slide was covered with 95% alcohol for 10 seconds and washed with tap water and drained. The slide was then flooded with safranin solution and allowed to counterstain for 30 seconds and was washed off with tap water, drained and blotted dry with filter paper and then examined under the microscope using the oil immersion lens. Biochemical test was also carried out to help identify isolates. The following tests were performed.

Coagulase test

A drop of distilled water was placed on each end of a slide. A colony of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was then added to one of the suspensions and mixed gently. The suspension on each side of the slide was observed for clumping of the organisms within 10 seconds. No plasma was added to the second suspension to help differentiate any granular appearance of the organism from true coagulase clumping.

Indole test

The test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water and Incubated at 35–37 0C overnight. Indole production was then tested by adding 0.5 ml of Kovac's reagent and shook gently then examined for a red color in the surface layer within 10 minutes.

Catalase test

3 ml of hydrogen peroxide solution was poured into a test tube. A sterile glass rod was used to remove several colonies of the test organism and immersed in the hydrogen peroxide solution. The test tube was then observed for immediate bubbling.

Statistical analysis

Semen analysis reports were checked for completeness and kept safely to guarantee confidentiality. Results were coded, entered into SPSS version 16 software and analyzed. Descriptive statistics were done utilizing frequencies and percentages and results were displayed using tables and graphs. The prevalence of bacteriospermia was computed from the extent of positive cases to the number of study population and presented as percentages.

RESULTS

The average age of the study participants was 40.5 years, with age group 35-44 years (42.3%) being the most represented group, followed by 25-34 years (36.7%), 45-54 years (18.7%), 55-64 year (1.7%) and 65-74 year (0.7%). Higher proportion (94.3%) of semen sample was by masturbation while 5.7% was by coitus interruptus. Majority (94.3%) of the samples were obtained from the center and few were brought to the center from participant's residence (Table 1).

Table 1: Age range, method and place of semen collection.

Variable	Frequency (n)	Percent
Age groups (years)		
25-34	110	36.7
35-44	127	42.3
45-54	56	18.7
55-64	5	1.7
65-74	2	0.7
Place of sampling		
Home	15	5.0
Centre	285	95.0
Methods of sampling		
Coitus interruptus	17	5.7
Masturbation	283	94.3

One hundred and twenty (40%) of the samples had prolonged liquefaction time whiles one hundred and eight (36%) liquefied at the normal liquefaction time. Rapid liquefaction was observed in 72 (24%) samples. One hundred and twenty-three (41%) were highly viscous, seventy-two (24%) were of low viscosity and the remaining hundred and five (35%) were of normal viscosity (Table 2). 40 (13.3%) of the participants produced semen volume of less than 1.5 ml, 95 (31.7%) of the samples were of high volume and the remaining 165 (55%) samples were within the normal volume (Table 2). Out of the total of 300 respondents, 21 (7%) produced samples with no spermatozoa (azoospermia), 120 (40%) produced samples with sperm count of less than 39 million, while 159 (53%) samples had a sperm count of ≥ 39 million. A total of 209 (69.7%) out of 300 samples had sperm of normal morphology of ≥ 4% while 70 samples (23.3%) had sperms of abnormal morphology (Teratozoospermia).

The most identified defects included head abnormalities, which was 60%, of which 10% had amorphous heads, 15% had large heads, 15% had thin heads, and 20% had double heads, neck abnormalities amounted to 30%, of which bent necks represented 12%, swollen necks 3% and broken necks 15%. Tail abnormalities was observed among 10% of which 2% were short tails, 5% were coiled tails and 3% double tails.

Out of a total of 300 samples, 246 (82%) produced semen with motile sperms while 33 (11%) had semen with spermatozoa that were completely immotile in ejaculate. No spermatozoa were observed in 21 (7%) of the semen samples. Out of the 246 (82%) samples that had motile sperms, 143 (47.7%) of the samples had spermatozoa of Progressive Motility Rate of < 25 μm s−1) which is

considered to be rapidly progressive, 83 (27.6%) of the samples had sperms with sluggish progression, 20 (6.7%) of the samples had spermatozoa with non-progressive motility and 33 (11%) of samples had spermatozoa in ejaculates that were entirely non-motile (necrospermia).

Of the total samples processed, 21 (7%) had no spermatozoa (azoospermia), and 118 (39.3 %) had sperm concentration fewer than 15x106/ml (oligospermia), and the remaining 161 (53.7%) samples had a sperm concentration of greater than 15x106/ml (Table 2).

Table 2: Semen characteristics.

Parameters	Results					
Liquefaction time (min)	≤30			31-60	60-120	Total
	72 (24%)			108 (36%)	120 (40%)	300 (100%)
Viscosity	Highly viscous			low viscosity	Normal viscosity	Total
	123 (41 %)			72 (33.3%)	105 (31.7%)	300 (100%)
Volume (ml)	≤1.5 ml			1.5-5 ml	>5ml	Total
	40 (13.3%)			165 (55%)	95 (31.7%)	300 (100%)
Total sperm count (10^6)	≤39			> 39	No spermatozoa	Total
	120 (40%)			159 (53%)	21 (7%)	300 (100%)
Sperm concentration (mill/ml)	≤15			> 15	No spermatozoa	Total
	118 (39.3%)			161 (53.7%)	21 (7%)	300 (100%)
Morphology (%)	≤4			> 4	No spermatozoa	Total
	70 (23.3%)			209 (69.7%)	21 (7%)	300 (100%)
Motility (%)	Motile			Non-motile	No spermatozoa	Total
	246 (82%)			33 (11%)	21 (7%)	300 (100%)
Progression	Rapidly progressive (>25μms^{-1})	Sluggish progression (5-25μm s^{-1})	Non-progressive Motility	Immotility	No spermatozoa	Total
	143 (47.7%)	83 (27.6%)	20 (6.7%)	33 (11%)	21 (7%)	300 (100%)

Of the total samples processed, 30 (10%) were found to be normospermic, 21 (7%) had no spermatozoa (azoospermia), and 118 (39.3 %) had sperm concentration fewer than 15x106/ml (oligospermia).

Teratozoospermia was identified among 70 (23.33 %) of the total samples processed while 33 (11%) of the samples were asthenozoospermic.

Oligoteratozoospermia and oligoasthenozoospermia were each identified in 9 (3 %) of the samples, 4 (1.3 %) of the samples were asthenoteratozoospermic, 6 (2%) of the samples were oligoasthenoteratozoospermic (Table 3).

Table 3: Semen abnormalities.

Type	Frequency (n)	%
Normospermia	30	10
Azoospermia	21	7
Oligozoospermia	118	39.33
Teratozoospermia	70	23.33
Asthenozoospermia	33	11
Oligoteratozoospermia	9	3
Oligoasthenozoospermia	9	3
Asthenoteratozoospermia	4	1.3
Oligoasthenoteratozoospermia	6	2

Table 4: Distribution of bacteria in semen samples

Organism	Frequency (n)	%
E. coli	27	40.3
S. aureus	13	19.4
U. urealyticum	10	14.9
Chlamydia trachomatis	9	13.4
Pseudomonas spp	5	7.5
Proteus spp	1	1.5
Klesbsiella spp	1	1.5
M. morganii	1	1.5
Total	67	100

Only 67 (22.3%) samples of the total of 300 samples, showed significant growth of bacteria of between 1×103 CFU /mL to 3×103 CFU/ ml. The remaining 233 (77.7%) showed counts less than 1× 103 CFU/mL, which is considered as negative cultures according to WHO criteria.[11]

Eight bacterial species were isolated. These include *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Ureaplasma urealyticum (U. urealyticum), Chlamydia trachomatis, Pseudomonas spp, Proteus spp, Klesbsiella spp* and *Morganella morganii (M. morganii).*

E. coli was isolated in 27 (40.3%) samples, 13 (19.4%) of the samples yielded *Staphylococcus aureus*, 10 (14.9%) yielded *U. urealyticum*, 9 (13.4%) yielded *C. trachomatis*, 5 (7.5%) yielded *Pseudomonas spp*., and 1 (1.5%) each yielded *Proteus spp*, *Klesbsiella spp*, and *M. morganii* (Table 4).

Of the 27 semen samples from which *E. coli* was isolated, thirteen (48.2%) were isolated from asthenozoospermic samples, five (18.5%) from teratozoospermic samples, four (14.8%) from astheoteratozoospermic samples, and two (7.4%) each from azoospermic and olgoasthenoteratozoospermic samples, and 1 (3.7%) from oligoteratogenic sample. E. coli was not isolated in any of the normospermic, oligozoospermic, and oligoasthenozoospermic samples. Of the 13 semen samples from which is *S. aureus* was isolated, 6 (46.2%) were from oligozoospermic samples, 3 (23.1%) were from oligoteratozoospermic samples, 2 (15.4%) were from teratozoospermic samples, and 1 (7.7%) each from asthenozoospermic and oligoasthenoteratozoospermic samples. Out of the 10 semen samples from which *U. urealyticum* was isolated, 4 (40%) each were from oligozoospermic and oligoteratozoospermic samples while 2 (20%) were isolated from teratozoospermic semen samples. 6 (66.7%) out of the 9 semen samples from which C. trachomatis was isolated were oligospermic, 2 (22.2%) were oligoteratozoospermic, and 1 (11.1%) was teratozoospermic. 2 (40%) out of 5 semen samples from which *Pseudomonas spp* was isolated 2 (40%) were normospermic whiles the remaining 3 (60%) were asthenozoospermic. With the exception of S. aureus, all the other isolates were Gram negative (Table 5).

Table 5: Semen abnormalities and kind of bacteria isolates identified.

Type	E. coli (%)	S. aureus (%)	U. urealyticum (%)	C. trachomatis (%)	Pseudomonas spp (%)	Proteus spp (%)	Klesbsiella spp (%)	M. morganii (%)
Normospermia	-	-	-	-	2 (40)	1 (100)	-	-
Oligozoospermia	-	6 (46.2)	4 (40)	6 (66.7)	-	-	-	1 (100)
Azoospermia	2 (7.4)	-	-	-	-	-	1 (100)	-
Teratozoospermia	5 (18.5)	2 (15.4)	2 (20)	1 (11.1)	-	-	-	-
Asthenozoospermia	13 (48.2)	1 (7.7)	-	-	3 (60)	-	-	-
Oligoteratozoospermia	1 (3.7)	3 (23.1)	4 (40)	2 (22.2)	-	-	-	-
Asthenoterato-zoospermia	4 (14.8)	-	-	-	-	-	-	-
Oligoastheno-zoospermia	-	-	-	-	-	-	-	-
Oligoasthenoterato-zoospermia	2 (7.4)	1 (7.7)	-	-	-	-	-	-
Total	27 (100)	13 (100)	10 (100)	9 (100)	5 (100)	1 (100)	1 (100)	1 (100)

DISCUSSION

The study analysed the quality of the semen and the presence of infection of male partners of infertile couples attending a fertility clinic. One hundred and twenty (40%) of the samples had prolonged liquefaction time whiles one hundred and eight (36%) liquefied at the normal liquefaction time. Rapid liquefaction was observed in 72 (24%) samples. One hundred and twenty-three (41%) were highly viscous, seventy-two (24%) were of low viscosity and the remaining hundred and five (35%) were of normal viscosity (Table 2). These figures are high compared to the results of other studies.18 Altered consistency has been attributed to altered chemical composition of the seminal plasma. The too thick and too light specimens have lower sperm concentrations than those with normal consistency.[19] In present study, 40 (13.3%) of the participants produced semen volume of less than 1.5 ml (Table 2), a figure lower than that of findings of Ramesh et al., and Jajoo et al., but higher than that of Enwuru et al.[18] 95 (31.7%) of the samples were of high volume and the remaining 165 (55%) samples were within the normal volume.[19,20] In one study, 77% had volumes within the normal range of 2-4 ml and only 1 (1%) had volume between 4-6 ml, 20 whiles another study had 87% producing volumes within the normal range and 7.7% producing higher volumes.[18] The low sperm volume could be as a result of spillage or sexual incontinence.[19,21] Low volume semen can result in low sperm count and low motility, parameters which are very necessary for fertilization. On the other hand, high volume semen could result in over-dilution of the sperm cells, hence low sperm concentration.[19]

Twenty-one semen samples (7.0%) were azoospermic, 118 (39.3%) had spermatozoa ≤ 15 million spermatozoa per ml (oligospermia) while 161 samples (53.7%) had spermatozoa >15 million per ml (Table 2). This higher

number of samples with good sperm concentration is contrary to the findings of other studies which reported only 11.5% semen samples of normal concentration.[22] Azoospermia recorded in this study could either be as a result of total obstruction of the seminal tract or testicular failure due to endocrine disorder.[23] Increased sperm concentration is associated with prolonged abstinence while improved motility is associated with shorter period of abstinence but with lower sperm density.[19] Out of the total of 300 respondents, 21 (7%) produced samples with no spermatozoa (azoospermia), 120 (40%) produced samples with sperm count of less than 39 million, while 159 (53%) samples had a sperm count of ≥ 39 million. A total of 209 (69.7%) out of 300 samples had sperm of normal morphology of ≥ 4% while 70 samples (23.3%) had sperms of abnormal morphology (Teratozoospermia). The most identified defects included head abnormalities, which was 60%, of which 10% had amorphous heads, 15% had large heads, 15% had thin heads, and 20% had double heads, neck abnormalities amounted to 30%, of which bent necks represented 12%, swollen necks 3% and broken necks 15%. Tail abnormalities was observed among 10% of which 2% were short tails, 5% were coiled tails and 3% double tails. Out of a total of 300 samples, 246 (82%) produced semen with motile sperms while 33 (11%) had semen with spermatozoa that were completely immotile in ejaculate.

No spermatozoa were observed in 21 (7%) of the semen samples. Out of the 246 (82%) samples that had motile sperms, 143 (47.7%) of the samples had spermatozoa of Progressive Motility Rate of < 25 μm s−1) which is considered to be rapidly progressive, 83 (27.6%) of the samples had sperms with sluggish progression, 20 (6.7%) of the samples had spermatozoa with non-progressive motility and 33 (11%) of samples had spermatozoa in ejaculates that were entirely non-motile (necrospermia). Of the total samples processed, 21 (7%) had no spermatozoa (azoospermia), and 118 (39.3 %) had sperm concentration fewer than 15x106/ml (oligospermia), and the remaining 161 (53.7%) samples had a sperm concentration of greater than 15x106/ml (Table 2).

Male infertility can be caused by problems in sperm DNA, even when count, motility, and morphology look normal. Although estimates vary, approximately 15% of patients with male factor infertility have normal spermiograms.[24]

There is evidence to support that integrity of sperm DNA influences a couple's fertility and helps predict the chances of pregnancy and its successful outcome.[24,25] Of the total samples processed, 30 (10%) were found to be normospermic, 21 (7%) had no spermatozoa (azoospermia), and 118 (39.3 %) had sperm concentration fewer than 15x106/ml (oligospermia). Teratozoospermia was identified among 70 (23.33 %) of the total samples processed while 33 (11%) of the samples were asthenozoospermic. Oligoteratozoospermia and oligoasthenozoospermia were each identified in 9 (3 %) of the samples, 4 (1.3 %) of the samples were asthenoteratozoospermic, 6 (2%) of the samples were oligoasthenoteratozoospermic (Table 3).

Only 67 (22.3%) samples of the total of 300 samples, showed significant growth of bacteria of between 1×103 CFU /mL to 3×103 CFU/ mL. The remaining 233 (77.7%) showed counts less than 1× 103 CFU/mL, which is considered as negative cultures according to WHO criteria.[11] This is lower compared to the results of other studies, but higher than that of others. [18,19,22,26-31] Eight bacterial species were isolated. These include *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Ureaplasma urealyticum (U. urealyticum), Chlamydia trachomatis, Pseudomonas spp, Proteus spp*, *Klesbsiella spp* and *Morganella morganii (M. morganii). E. coli* was isolated in 27 (40.3%) samples, 13 (19.4%) of the samples yielded *Staphylococcus aureus*, 10 (14.9%) yielded *U. urealyticum*, 9 (13.4%) yielded *C. trachomatis*, 5 (7.5%) yielded *Pseudomonas spp*., and 1 (1.5%) each yielded Proteus spp, Klesbsiella spp, and M. morganii (Table 4).

E. coli, followed by *S. aureus* were the most predominant bacteria isolates in this study. A finding similar to that of others, but contrary to that of others, where *S. aureus*, followed by *E. coli* were the most dominant bacterial isolates.[19,21,30,32,33] The most frequently isolated bacteria in this study (*E. coli, S. aureus, U. urealyticum, Chlamydia trachomatis, Pseudomonas sp*p) have been shown to negatively impact on fertility. *E. coli, U. urealyticum* and *S. aureus* have been shown to negatively affect the reproductive potential of sperm.[34] *E. coli* at high concentration has been found to inhibit progressive motility of sperm.[35,36] It does this by adhering to sperm and causing them to agglutinate, subsequently destroying the sperm membrane.[35] In this study 48.2% of the samples from which E coli was isolated were asthenozoospermic. *E. coli* and *S. aureus* are known to induce apoptosis/necrosis in spermatozoa when incubated together.[37] Genital mycoplasmas and ureaplasmas seem to be widespread among infertile male patients, and U. urealyticum infection is considered as one of the causes of male infertility.[12,38] The strong adhesive properties of these microbe's cause agglutination of sperm which may be related to diminished cell motility. The most toxic agents for spermatozoa are the metabolic products of U. urealyticum. These include H_2O_2 and ammonia (NH3). Although H_2O_2 is itself harmful to sperm, its product, hydroxide anion ($OH^{\cdot}$), is a highly toxic radical for cell membranes. Furthermore, Ureaplasma phospholipases A and C may influence changes in the lipid composition of the cell membranes of male gametes, leading to loss of integrity and increased permeability.[12]

The study showed that most of the male partners of infertile couples attending the fertility clinic had abnormal semen quality, with oligozoospermia being the predominant semen anomaly. Bacteria were isolated from 22.3% of the semen samples. *E. coli* was the most

isolated bacterium. These bacteria could be responsible for the poor semen and sperm quality which results in male infertility. Even when count, motility, and morphology look normal other parameters such as infection and sperm DNA should be investigated during the treatment of infertility.

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