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The effect of presence of facultative bacteria species on semen and sperm quality of men seeking fertility care

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Abstract

Introduction: Infections of male urogenital tracts may contribute to male infertility. However, the effects of bacterial presence on sperm quality and fertility are controversial.

Objectives: We investigated the occurrence of non-specific bacteria and quality/quantity of semen of infertile and fertile control groups in Nigeria.

Subjects and methods: We investigated 162 infertile and 54 fertile men. Spermiogram, culture, bacterial isolation and characterization were conducted.

Results: We report 114/162(70.4%) occurrence of bacteria species, 49.4% of such were Gram positive and 21% Gram negative: *Staphylococcus aureus* (29.6%) and *Escherichia coli* (10.5%) had the highest occurrence for each group respectively. On semen quality/quantity, we report 14.2% azoospermia, 52.5% oligozoospermia and 33.3% of normozoospermia. The mean sperm concentrations were 10×7/ml and 41×10 6/ml for oligo and normozoospermia respectively. Majority (52%) of azoospermic group had no bacterial growth. *S. aureus* was the most implicated among the bacterial positive group. Within the ologozoospermic category, 28% had no bacterial growth, 28% had *S. aureus* and 11.8% *E. coli*. The normozoospermic patients had 18.5% no bacteria contamination, 33.3% had *S. aureus*, 13% had *E. coli*. From the analysis, the normozoospermic group with bacterial contamination had lower sperm concentrations compared with those without contamination. It was apparent that factors other than bacterial contamination may contribute more to oligozoospermia (compare: "no bacteria" group mean sperm concentration 8.97×10^6/ml, Gram positive bacteria contaminated group 17.74×10^6/ml and Gram negative bacteria

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contaminated group 13.66 × 106/ml). The mean progressive motility ratios were lower (15.6 [a]% + 18.3 [b]%) = 33.9%) against WHO standard (a + b = >50%) and control RPM (a) = 55.3%. Generally, the semen quality (vol., rapid progressive motility, sperm concentration and immotility) were significantly lower than the fertile group, $P = 0.0005$, <0.0001, <0001 and 0.0335, respectively.

Conclusions: Although bacterial presence in semen reduced mean sperm concentration and viability, thereby contributed to oligozoospermia and by extension the chances of siring a child, however, factors other than bacterial presence may contribute more. Improved interpretative approaches of semen analyses are highlighted.

Introduction

Infertility constitutes medical and socio-cultural problems globally [1], and bacterial infections contribute to about 15% of male infertility [2]. The WHO study Centre in Africa reported 50% of male contributory factors of infertility [3,4], and 46% of those reported history of sexually transmitted infections [4].

Infections of the male genitourinary tract may contribute to infertility by adversely affecting sperm function, causing inflammatory disorder, anatomical obstruction, scaring and initiating leucocyte response with its concomitant oxidative stress. The effects of these conditions may be sperm damage, elevated leucocyte response (pyospermia), poor motility (asthenospermia) and immature forms (tetratospermia) [5]. The remote effect usually is low sperm quality and hence male infertility [5–8].

Microbial genital tract infections could be specific (Chlamydosis caused by *Chlamydia trachomatis*, gonorrhoea caused by *Neisseria gonorrhoea*, ureaplasmosis caused by *Ureaplasma urealyticum* and trichomoniasis caused by *Trichomonas vaginalis*) and non-specific (facultative) aetiology (mainly by: *Enterobacteriaceae* e.g. *E. coli*), *Staphylococci, Streptococci, Klebsiella spp.*, and yeast-like cells (a fungus) [9,10].

One of the most frequently isolated microorganism from male patients with genital tract infections or semen contamination is *Escherichia coli* [11]. The negative influence (qualitative and quantitative alterations) of this species on sperm quality was associated partially to its effect on motility [11] and to the impaired acrosomal function, as demonstrated at the ultrastructural level by Diemer et al. [12]. The influence of Gram-positive uropathogenic bacteria on sperm morphology and function has been poorly investigated until now. Mehta et al. [13] reported that aerobic cocci are present in about 50% of semen samples of male partners in infertile couples. *Enterococcus faecalis* was isolated from 53% of patients, *micrococci* from 20% and alpha-haemolytic *streptococci* from 16% of the infected samples. Increased prevalence of genital tract infections caused by *E. faecalis* was associated with compromised semen quality in terms of sperm concentration and morphology and the presence of *micrococci* and alpha-haemolytic *streptococci* does not appear to exert any detrimental effect on sperm quality [14]. Although no significant depressor effect of *enterococci* on sperm motility was observed, some researchers described, in an in vitro study, a negative influence on membrane integrity of human sperm head, neck and mid piece [14], probably mediated by hemolysin, a well-known virulence factor of *enterococci*.

While primary infertility was reported higher in other regions of the world, secondary infertility was reported as more common in Africa [9]. The reasons adduced to this included, but not limited to: inadequate health care services, improper use of antibiotics and the new trends in drug resistance [9].

Although, positive correlations between azoospermia, presence of microorganisms and poor semen quality and quantity in male patients have been reported [10,15] the interplay between semen bacterial contamination and male infertility is still subject to controversy, since some reports on the presence of bacteria in semen specimens of infertile men had somewhat similar occurrence to those observed in some fertile males [8,16].

Knowledge of certain sexual and fertility conditions such as oligzoospermia, azoospermia, impaired sperm motility, decreased sexual drive, weak erection, and premature ejaculation are critical for primary evaluation of infertility in men. Semen analysis is not a test for fertility because normal values are subjective and have been difficult to determine for fertile and infertile men. However, clinical studies have tried to establish "limits of adequacy" using semen analysis below which the chances of initiating a pregnancy may become more difficult. These parameters are not absolute because some fertile men may have values below these "limits of adequacy". Conversely, some infertile men have normal semen parameters by standard analysis and that in most cases the result interpretations are subjective due to lack of adequate and unified information on the roles of nonspecific microbes globally. In Africa, available studies are inadequate. Therefore, in other to make semen analysis more efficacious, give more statistical interpretations to certain semen parameters and, assess the role of facultative bacteria in alterations of the integrity of human reproductive system, this study was designed to investigate the occurrence of facultative bacteria contaminants in semen and their possible effect on quality and quantity of sperm cells among men attending fertility clinic in Lagos, Nigeria.

Subjects and methods

Patient's recruitment

One hundred and sixty-two (162) out of 566 patients attending selected fertility clinics in Lagos, between 2006 and 2013 and had regular unprotected sex with their spouses without conception for between 1 and 2 years and above were involved in the study.

Clinical and physical examinations were previously conducted on the patients by the urologist prior to the patients' visit to the laboratory. Those with the following exclusion criteria were not included in this report: altered hormonal level, recent or ongoing antibacterial treatment, anatomical problems (varicocele, cryptorchidism etc.), also, those with obvious sperm defects of suspected genetic reasons and alterations affecting vast majority of the sperm cells population and patients with multiple bacterial infections.

For those who qualified, spermiogram and semen culture were routinely performed on their specimens.

Control group

Fifty-four specimens from fertile men (having sired at least a child in the last 2 years), aged 20–60 years with normal karyotype, had no anatomical challenges and no symptomatic bacterial infection were used as control.

The ethical clearance was obtained from NIMR IRB and both patients and control participants gave a written informed consent before recruitment.

Semen sample collection

Continence of 3–7 days was observed by each patient who was previously counselled to wash the hands, penis and scrotum before ejaculation to avoid bacterial cross contamination. Ejaculation was by masturbation.

Sample processing

Specimens were examined after liquefaction for 30 min at 37 °C, volume, sperm concentration; pH, sperm motility and leucocyte presence were evaluated according to WHO standard [17].

Culture procedure

The semen were allowed to liquefy completely and then inoculated as: undiluted, 1:10 diluted and 1:100 diluted samples using standard loop on agar plates [18,19]. Briefly, specimens were cultured onto: Nutrient, MacConkey, Chocolate and Blood agar. Samples were incubated in a microaerophilic (5% CO_2) and aerobic conditions at 37 °C overnight [19].

Seminoculture were considered positive when the number of colonies was $\geq 10^4$ CFU ml^{-1} for Gram positive cocci and $\geq 10^5$ CFU ml^{-1} for Gram negative rods [17].

Characterization

Microorganisms were identified by Gram staining, oxidase, catalase and other sugar utilization biochemical tests [18].

Culture for strict anaerobes was not part of this study design and was not carried out.

Statistical analysis.

Data analysis was done using SPSS version 15 statistical package. Participants with bacterial isolates were grouped according to the bacterial species. Semen parameters and characteristics
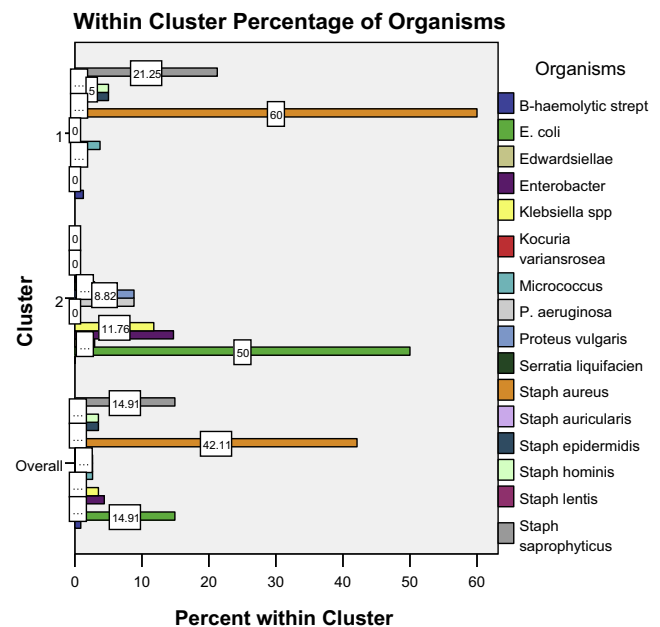

Figure 1 Graphical representation of the prevalence of bacterial species isolated. Key: cluster 1 = Gram positive organisms. 2 = Gram Negative organisms.

were expressed as percentages, means and standard deviation. Skewedness (lack of symmetry) and kurtosis (measure of peak or flatness of data in relation to normal distribution) were carried out. Within cluster variation effects and Cluster wise importance of presence or absence of bacteria were carried out. Two tailed t-test were carried out on data with normal distributions, P-values < 0.05 were considered statistically significant at 95% confidence interval.

Results

Out of 566 men who attended the fertility clinics, 162 participants (age between 21 and 50: mean 39 years) met the inclusion criteria. Of this, 114 (70.4%) had bacterial isolates. The prevalence of bacteria species were as shown in Fig. 1.

Eighty (49.4%) of all the isolates were Gram positive. *Staphylococcus aureus* 48 (29.6%) had the highest occurrence. Thirty-four (21%) were Gram negative species and *E. coli* (10.5%) was the most implicated.

The overall mean sperm concentration of the patients studied was 18.83 million/ml (Azoo, oligo and normozoospermia). Twenty three (14.2%) were azoospermic, 85 (52.5%) oligozoospermic and 54 (33.3%) had normozoospermia. Excluding azoospermic population, the mean sperm concentration of 22 million cells/ml (mean oligozoospermia 10 million cells/ml and mean normozoospermia of 41 million cells/ml were record.

Fig. 2 shows graphical representation of sum total occurrences of each species of facultative microbial organisms isolated in relation with the mean sperm concentration.

Of the 23 patients with azoopermia, majority 12(52.2%) had no microbial growth, 6(26.1%) had *S. aureus*. One (4.34%) patient each
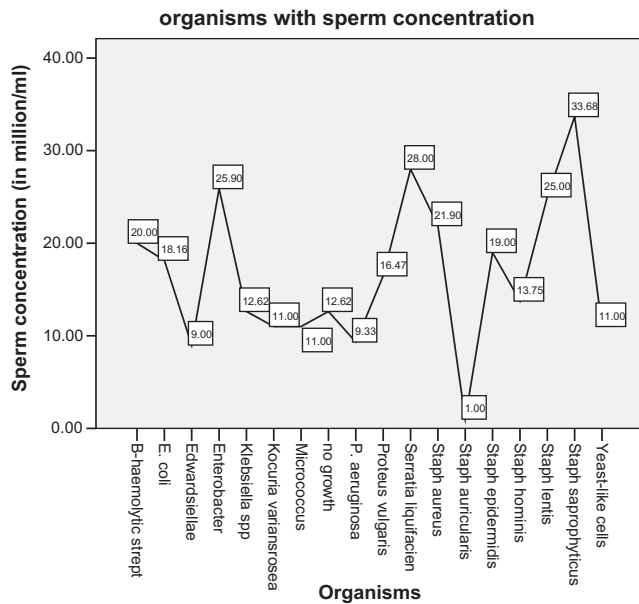
Figure 2   Graphical representation of organisms isolated with the sperm concentration.

had: *Klebsiella spp., P. aeruginosa*, *S. hominis* and *S. saprophyticus* respectively. One (4.34%) patient had yeast-like cells (a fungus).

Tables 1 and 2 show the frequency distributions based on the total population studied of specific microbial isolations for oligozoospermic and normozoospermic patients respectively.

Among the normozoospermic patients, 10/54 (18.5%) had no bacterial isolation, 18/54 (33.3%), 9/54 (16.7%) and 7/54 (13%) yielded *S. aureus*, *S. saprophyticus* and *E. coli* respectively among other species.

Applying statistical analysis of attribute of importance (SPSS version 15.0). Fig. 3 demonstrates within cluster variation of oligozoospermia with and without bacterial presence.

Observe that lower sperm concentration obtains among fertility seeking men without bacterial contamination. However, in general the patients studied with the presence of bacteria were more in population and contributed more to the overall oligozoospermic category.

Conversely, the case of normozoospermia group is as shown in Fig. 4.

Notice that patients with bacteria contamination presented with lower sperm concentration (cluster 2) considering the lower limit of >20 million cells/ml and were higher in population studied, this presupposes that absence of bacterium produced higher sperm concentration; but those with bacteria influenced more the lower mean sperm concentration reported for normozoospermia in this report.

Generally, the overall mean sperm concentration of the infertile population was oligozoospermic (<20 million sperm cells/ml); however, cluster segregations compared with the overall result are presented below in Fig. 5 and this shows the cluster influences associated with the sperm concentrations and their attributes of importance.
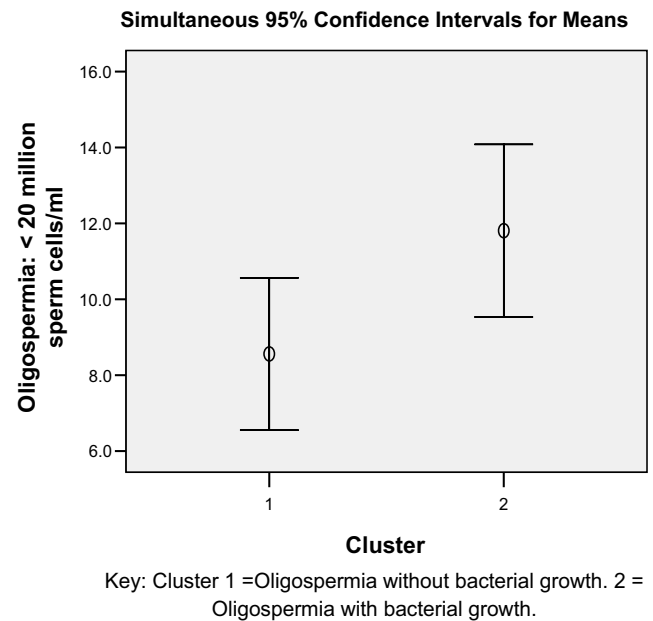

Key: Cluster 1 =Oligospermia without bacterial growth. 2 = Oligospermia with bacterial growth.

Figure 3   Within cluster variation effect of presence or absence of bacterial organisms on oligozoospermic sperm concentration category.
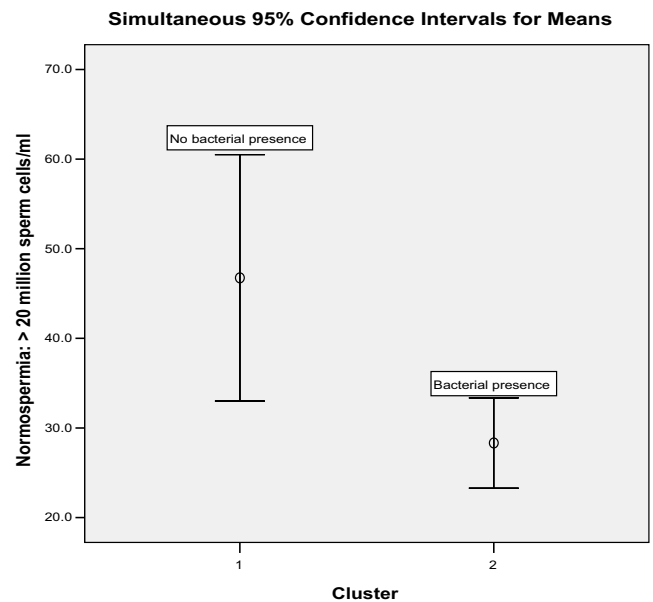

Figure 4   Within cluster variation effect of presence or absence of bacterial organism on normozoospermic sperm concentration.

To further buttress the strength of the influence of different clusters from the population studied, a "TwoStep Cluster Number Frequency" analysis is as shown in Fig. 6. The distribution is asymmetric and the pick between clusters 2 and 3.

Motility

On the whole, 139/162 (85.8%) fertility seekers had sperm cells in their semen samples; the motility rates were categorized as **a, b, c** and **d** for: rapid progressive, sluggish progressive, non-progressive motility and immotility, respectively. Considering the 23/162 (14.2%) azoospermic patients, approximately the mean

Table 1 The frequency of oligozoospermic specific organisms isolated based on total population studied.

Category & organism		Frequency	Percent	Valid percent	Cumulative percent
Valid	Azoo plus Normozoospermic[a]	77	47.5	47.5	47.5
	E. coli	**10**	**6.2**	**6.2**	**53.7**
	Edwardsiella	1	.6	.6	54.3
	Enterobacter	3	1.9	1.9	56.2
	Klebsiella species	2	1.2	1.2	57.4
	Kocuria varansrosea	1	.6	.6	58.0
	Micrococcus	3	1.9	1.9	59.9
	No growth	**24**	**14.8**	**14.8**	**74.7**
	P. aeruginosa	2	1.2	1.2	75.9
	Proteus vulgaris	2	1.2	1.2	77.2
	Staph aureus	**24**	**14.8**	**14.8**	**92.0**
	Staph auricuralis	1	.6	.6	92.6
	Staph epidermidis	3	1.9	1.9	94.4
	Staph hominis	2	1.2	1.2	95.7
	Staph saprophyticus	7	4.3	4.3	100.0
	Total	**162**	**100.0**	**100.0**	

[a] Total frequency for azoospermic and normozoospermic categories studied.

motility categories were: 29%, 20%, 9% and 42% for rapid progressive, sluggish progressive, non-progressive motility and immotility respectively.

Morphology

On the other hand the 85.8% of the patients with sperm cells had normal (≤30% abnormal sperm cells) mean sperm morphology rate of 48.3%, while mean abnormal (>30% abnormal sperm cells) morphology was put at 51.7%.

Analysis

Using a two-step-cluster analysis of the 114 (34 Gram negative and 80 Gram positive) patients with bacteria growth segregated along Gram reactions on one hand and the profiles of other variables on the another hand as follows: Sperm Concentration (SC), Abnormal Morphology (AM) and Motility [Non-Progressive Motility (NPM), Sluggish Progressive Motility (SPM) and Immotility

(I)]. Cluster 1 (Gram positive organisms) attribute of poor sperm quality mean rates of: 45.4 ± 27.9%, 18.8 ± 10.0%, 10.3 ± 11.5%, and 35.4 ± 20% for AM, SPM, NPM and I respectively; while cluster 2 (Gram negative organisms) mean rates of: 41.4 ± 29.3%, 17.3 ± 14.3%, 7.1 ± 9.5% and 38.1 ± 25.2% were recorded respectively for the same variables.

Fig. 7 shows the Gram reactions cluster effect on SC of the patients and Figs. 8 and 9 demonstrate same for I and SPM respectively.

Other seemingly relevant variables recorded for this study are: mean volume 2.77 ± 1.2 ml, 124 (76.6%) were milky, 11 (6.8%) clear and 27 (16.7%) were brown in colour. On consistency of the semen studied, 57 (35.2%) were viscous and opaque, 32 (19.8%) translucent and majority 73 (45.1%) were watery and turbid. pH reports had normal pH (7.2–8.0) in 143 (88.3%), <7.2 in 10 (6.2%) patients and 9 (5.6%) pH > 8.0. On white blood cells, 117 (72.2%) had WBC less than 1×10^6/ml and only 45 (27.8%) had WBC $\geq 1 \times 10^6$/ml.

Table 2 The frequency of normozoospermic specific organisms isolated based on the total population studied.

Category & organism		Frequency	Percent	Valid percent	Cumulative percent
Valid	Azoo plus Oligospermic[a]	108	66.7	66.7	66.7
	B-haemolytic streptococcus	1	.6	.6	67.3
	E. coli	**7**	**4.3**	**4.3**	**71.6**
	Enterobacter	1	.6	.6	72.2
	Enterobacter	1	.6	.6	72.8
	Klebsiella species	1	.6	.6	73.5
	No growth	**10**	**6.2**	**6.2**	**79.6**
	Proteus vulgaris	1	.6	.6	80.2
	Serratia liquifacien	1	.6	.6	80.9
	Staph aureus	**18**	**11.1**	**11.1**	**92.0**
	Staph epidermidis	1	.6	.6	92.6
	Staph hominis	1	.6	.6	93.2
	Staph lentis	1	.6	.6	93.8
	Staph saprophyticus	9	5.6	5.6	99.4
	Yeast-like cells	1	.6	.6	100.0
	Total	**162**	**100.0**	**100.0**	

[a] Total frequency for azoospermic and oligozoospermic categories studied.
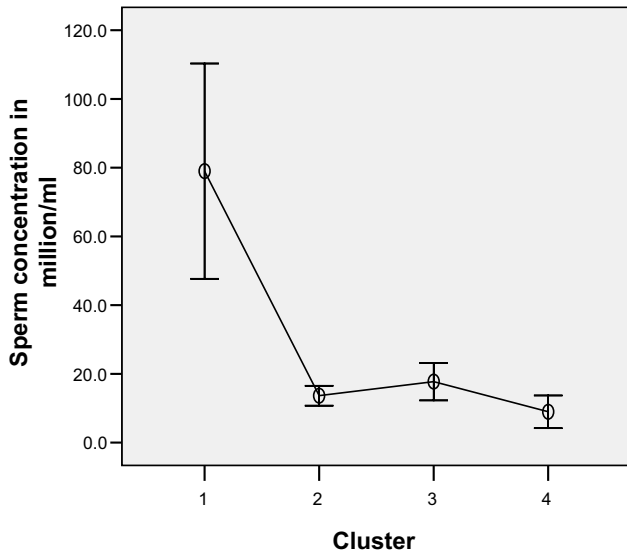
Figure 5 Graph evaluating sperm concentration categories and presence or absence of microorganism among fertility care seeking men studied. Key: Custer 1: = 12 patients with Gram positive organisms plus 2 yeast like cells isolated (14 patients) with normozoospermia; mean sperm concentration 79 million/ml (±40.5 SD). Cluster 2 = 68 patients with Gram positive organisms with oligozoospermia mean concentration 13.66 million/ml (±9.27). Cluster 3 = 34 patients with Gram negative isolates mean oligozoospermia 17.74 million/ml (±12.04). Custer 4 = 46 patients with no bacterial isolates, mean oligozoospermia 8.97 million/ml (±12.39).
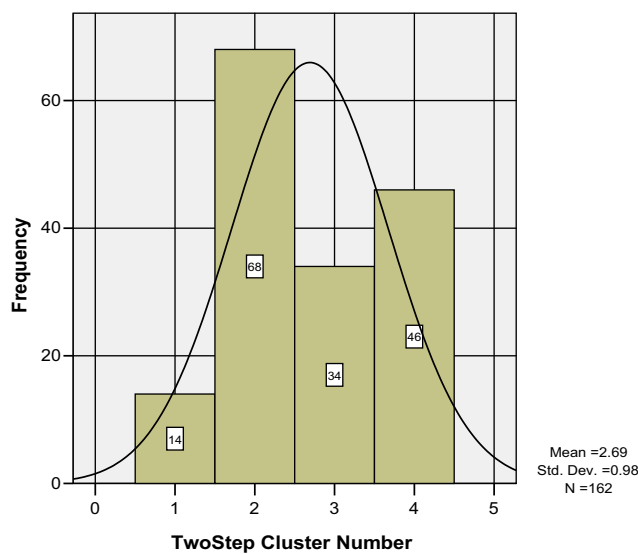

Figure 6 Frequency histogram demonstrating clusters' Skewedness and kurtosis.

Control group

Conversely, data from the 54 control group recruited had mean statistics as follows: sperm concentration 73.3×10^6/ml, volume 3.4 ml, RPM 56.9%, SPM 20.7%, immotility 17.1%, normal
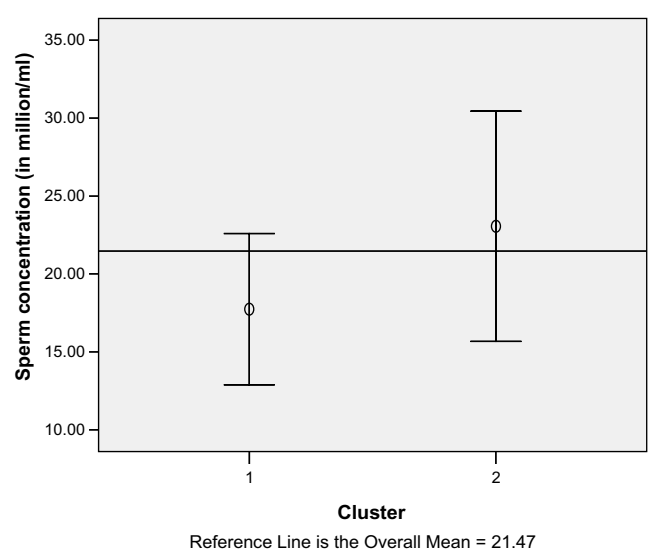

Figure 7 Within cluster variation of sperm concentration and cluster 1 (all Gram positive organisms) and cluster 2 (all Gram negative organisms) isolated from fertility care seeking men.
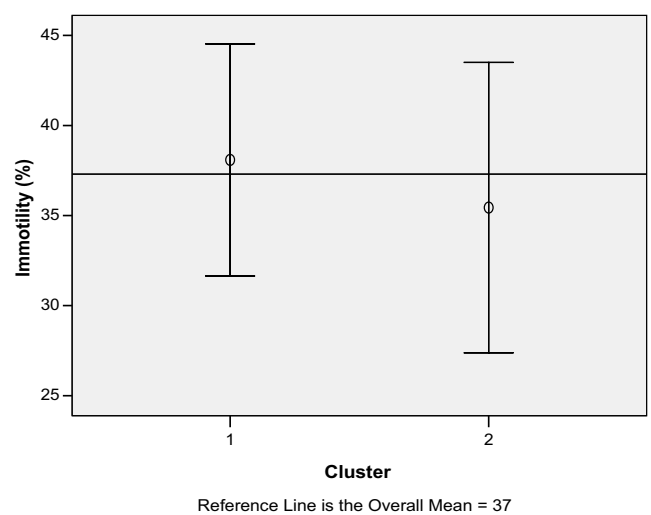

Figure 8 Within cluster variation of immotility, cluster 1 (all Gram positive organisms) and cluster 2 (all Gram negative organisms) isolated from fertility care seeking men.

morphology 77.5% and abnormal form 22.5%. Microbial quality screening yielded: no growth in 19 participants, *E. coli* (2), *Enterobacter* (4), *Micrococcus* (1), *Proteus vulgaris* (2), *S. aureus* (8), *S. epidermis* (2), *S. saprophyticus* (10) and yeast-like cells (6).

The comparative analysis of certain essential variables among study population and control group are as contained in Table 3 below.

Discussion

Semen analysis comprises of a set of descriptive measurements of spermatozoa and seminal fluids parameters that help to estimate semen quality [20]. Conventional basic semen analysis includes

Table 3 Means ± standard deviation of considered variables and specific bacterial organisms in each group: infertile versus fertile populations.

Variables	Organisms										*P*-value
	Test vs control No growth (46, 19)	Test vs control *E. coli* (17, 2)	Test vs control *Enterobacter* (5, 4)	Test vs control *Micrococcus* (3, 1#)	Test vs control *Proteus vulgaris* (3, 2)	Test vs control *S. aureus* (48, 8)	Test vs control *S. epidermidis* (4, 2)	Test vs control *S. saprophyticus* (17, 10)	Test vs control Yeast like cells (fungus) (2, 6)	Total test vs control (162, 54) Mean	
Vol. (ml) ± SD	3.0 ± 1.2	2.8 ± 0.94	2.9 ± 0.74	3.5 ± 1.5	4.0 ± 1.0	2.5 ± 1.2*	2.1 ± 0.85	3.0 ± 1.3	2.8 ± 1.3	2.8 ± 1.2	0.0005
	3.2 ± 0.51	2.8 ± 0.94	3.8 ± 0.29	2.0 ± 0	3.0 ± 0	3.6 ± 0.37	3.0 ± 0	3.6 ± 0.9	3.7 ± 0.26	3.4 ± 0.6	
RPM (%) ± SD	15.6 ± 20***	35.9 ± 16.1	37 ± 12.0***	23.3 ± 25.2	21.7 ± 14.4	25.3 ± 21.3**	30.0 ± 46.3	36.4 ± 21.2***	10.0 ± 14.6**	24.8 ± 20.8	<0.0001
	55.3 ± 10.5	60.0 ± 0	67.5 ± 2.9	50.0 ± 0	40.0 ± 0	55.0 ± 39	70.0 ± 0	61.0 ± 10.2	52.0 ± 9.0	57.0 ± 10	
SPM ± SD	18.3 ± 22	20.0 ± 9	22.0 ± 10.4	18.3 ± 2.9	15.0 ± 15.0	14.2 ± 13.7	22.5 ± 15	24.4 ± 14.9	5.0 ± 7.0	17.7 ± 16.2	0.149
	22.4 ± 3.5	20.0 ±	12.5 ± 29	20.0 ± 0	20.0 ± 0	15.6 ± 10.8	20.0 ± 0	24.0 ± 10.2	23.0 ± 5.2	21.0 ± 7.2	
NPM (%) ± SD	7.0 ± 14.5	10.9 ± 11.2	7.0 ± 9.7	3.3 ± 5.8	11.7 ± 10.4	7.4 ± 10.6	17.5 ± 6.5*	4.7 ± 6.0	7.6 ± 11.5	7.6 ± 11.5	0.0335
	3.7 ± 4.4	0.0 ± 0	5.0 ± 0	10.0 ± 0	10.0 ± 0	3.1 ± 2.6	0.0 ± 0	2.0 ± 4.2	10.0 ± 0	4.17 ± 4.3	
Immotility ± SD	33.2 ± 30.7*	35.0 ± 15	35.0 ± 18.0	55.0 ± 32.8	51.7 ± 30.1	40.2 ± 26.7	30.0 ± 14.7	30.9 ± 21.7*	0.0 ± 0.0**	36.0 ± 26.0	0.1474
	17.6 ± 9.2	20.0 ± 0	15.0 ± 5.9	20.0 ± 0	30.0 ± 0	21.3 ± 11.6	10.0 ± 0	13.0 ± 5.4	15.0 ± 4.5	17.0 ± 8.5	
Sperm conc. M/ml ± SD	12.6 ± 22***	18.2 ± 10.8	25.9 ± 19.4	11.0 ± 7.0	16.5 ± 9.8	22 ± 26	19.0 ± 4.5*	33.7 ± 43*	35 ± 49.5	18.8 ± 31	<0.0001
	105.3 ± 139.2	30.5 ± 7.8	41.5 ± 14.6	26.0 ± 0	44.0 ± 1.4	33.6 ± 15.7	40.0 ± 8.5	108.5 ± 107.5	30.3 ± 99	73.0 ± 9.5	
Abnor. morphology ± SD	47.1 ± 38.3*	50 ± 29.2	32 ± 8.4	41.7 ± 24.7	65 ± 35	39.7 ± 31	75.5 ± 17*	39.2 ± 27.4	35 ± 49.5	43.8 ± 31.9	0.2121
	17.9 ± 12.5	35.4 ± 5.8	35.0 ± 5.0	30.0 ± 0	20.0 ± 0	28.3 ± 13.5	30.0 ± 0	19.0 ± 14.3	20.0 ± 13.4	23.0 ± 14	

Note: We considered different variables of test (infertile men) and fertile control group considering a normal distribution, using a *t-test*, P values <0.05 were considered statistically significant at 95% confidence interval. Key: # means not computable, *Mocrococcus* (one isolate), vs = versus.

* $P < 0.05$.
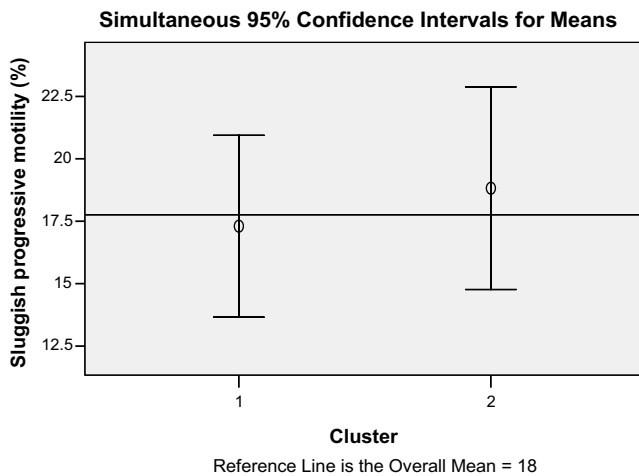** $P < 0.01$.
*** $P < 0.0001$.

Figure 9 Within cluster variation of sluggish progressive motility and cluster 1 (Gram positive) and cluster 2 (Gram negative) organisms isolated from fertility care seeking men.

but not limited to sperm concentration, motility and morphology [21]. Many scholars have worked on the uro-genital tract specific and facultative bacterial contamination in male infertility; however, the putative effect of these agents on the quality of semen is still controversial [8]. Presence of bacteria affect male reproductive function directly by impairing motility, causing agglutination, reducing the ability of acrosome reaction and alteration of sperm morphology, and indirectly causing scaring and producing oxidative stress through the release of Reactive Oxygen Species (ROS) [7,8]. However, reports on the presence of bacteria in semen specimens of infertile men has a similar occurrence to that observed in some fertile males [8,16] and therefore, calls to question the contribution of bacterial organisms on semen quality. Our observations in the present report were not expressly at variance either, however, for the very fact that absolute values of the semen analysis of asymptomatic apparently healthy fertile men had almost always remained superior to those seeking fertility care, there may be factors relating to either testicular abnormalities (excluded), body immunity or active/inactive presence of bacteria contributing to the poor semen quality. This made this present study relevant, qualitatively and quantitatively. In this report the mean sperm concentration of 18.8×10^6/ml was recorded for the infertile patients and was skewed towards oliozoospermia (52.5% with mean sperm concentration 10 million cells/ml) against the fertile control group with mean sperm concentration of 73.3×10^6/ml. The WHO normal range (normozoospermia) for fertile men is $20–120 \times 10^6$/ml [17]. Generally, the semen quality (volume, rapid progressive motility, sperm concentration and immotility) were significantly lower than the fertile group, $P = 0.0005$, <0.0001, <0001 and 0.0335 respectively Also, the mean progressive motility ratio were lower than that established by WHO guidelines (a + b) = >50%, where RPM = a and SPM = b or RPM alone >25% [17]. In this study, fertility seekers had (15.6% [a] + 18.3% [b]) = 33.9% and fertile control group had RPM[a] = 55.3%. Previous report by Moretti et al. [8] had 46.4% and 51% for RPM and SPM respectively on infertile men with presence of bacteria. Time and place of study may be a factor of variability.

This report shows that oligozoospermia and decreased sperm motility are common amongst male with fertility problem. We have demonstrated using cluster wise importance, that slow progressive motility were common with Gram positive organisms as a major factor contributory to poor sperm quality among this group, followed by abnormal morphology and this agrees with the report of Qiang et al. [14]. While, in Gram negative organisms essentially NPM was followed by SPM applying the same test. This report was explicated in a report by Moretti et al. [8] who suggested that bacterial flagella and pili (contact accessory structures) of *E. coli* and *M. morganni* could be an important determinant of pathogenicity. This was further supported by the work of Villegas et al. [22] who hypothesized that the mechanism of sperm damage caused by bacteria passes through the expression of the adhesive properties of the flagella and pili to mannose receptors. Also, mannose receptors have been demonstrated at the surface of human spermatozoa [23].

Specifically, *S. aureus* was the most implicated in azoospermic patients, while *S. aureus* and *E. coli* had the highest and second to the highest occurrence respectively among ologozoospermic group. However, same *S. aureus* had the highest occurrence followed by *S. saprophyticus* among normozoospermic patients. It had been reported that bacterial presence may affect the quality and quantity of semen by induction of apoptosis and necrosis [8]. Although Moretti et al. [8] did not isolate *S. aureus* but, the possible explanation of no significant effect report of some scholars on the fertile patients was that the bacterial contamination could possibly be recent and that the bacterial loads were low [22]. In this condition, the contact with bacteria had not been long enough to elicit production of substances that could damage sperm cells. Also we could suppose that the semen of fertile men was capable of hampering the potential mechanisms by which organisms may damage sperm cells [24]. From this study, it has been demonstrated that the sperm concentration of normozoospermic candidates with bacteria presence although had normal range of sperm concentration ($>20 \times 10^6$/ml), but had always been lower compared with those without bacterial presence, see Table 3.

S. aureus and *E. coli* have been reported to induce apoptosis in human sperm with two possible putative mechanisms: a direct cytotoxic activity of bacterial toxins and the contact with pili and flagella. *E. coli* in particular starts the apoptotic process by activating several caspases and proteases responsible for mitochondrial changes, alteration in membrane symmetry and DNA fragmentation and production of toxins and metabolic products originating from bacterial proliferation [22,24].

Further literature explanations resides on the existence of an antigenic mimicry between some constituent of sperm flagella such as tubulin found in axoneme, and bacterial proteins which may have pathogenic effect [8]. Infection may therefore induce antibodies and T-cells to react against bacterial cell constituents that may recognize self-components and immunomediated damage may follow. But simply, spermatozoa may share epitopes with bacteria (Antigens) of the most frequent species colonizing the genitourinary tract of man. The antigen may induce an antibody response which could cross-react with the flagella of spermatozoa affecting its life span and motility [24].

Several investigations which assessed in vitro fertilization indicated that oocyte fertilization was reduced in the presence of pathogenic organisms in semen [25] and concluded that semen bacteria contamination reduces semen quality, interferes with fertilization.

Diemer et al. [11], reported direct inhibitory effect of *E. coli* on progressive motility of spermatozoa and was found to depend upon the bacteria concentration. This finding is in agreement with this report of significant difference in RPM of studied population compared with that of control $P = < 0.0001$. Further observation by electron microscopy revealed multiple adhesion of *E. coli* to spermatozoa, causing variable ultrastructural damage as probable morphological damage correlates immobilization [11,26,27]. From this study, *E. coli* was found to be the most prevalent Gram negative organisms isolated among the oligozoospermic class of fertility care seekers. Although *E. coli* was found among the normozoospermic group, the bacterial population and the length of infection may vary [11].

From this present study, Fig. 7 demonstrates the order of importance of oligozoospermia and other possible variables. It shows that factors other than bacterial contamination contributed to low sperm concentration, hence 'no bacterial growth' group had the lowest grade of sperm cells compared to those with bacterial isolates. However, it is noteworthy that this work did not include isolation of strict anaerobic organisms.

Again, we observed that Gram negative organisms appear to exert more negative influence on quality of semen than Gram positive organisms using cluster attribute of importance; this is in line with the report of other scholars [28], who equally reported 'no association' of Gram positive organisms with degrading semen quality excepting enterococcus. However, various evidence abound to counteract 'a no effect' hypothesis: Huwe et al. [29] and Liu et al. [30] both agreed and found significant decrease in sperm motility when spermatozoa were incubated with *S. aureus*. In a more recent study, Gupta and Prabha [31], studied Sperm Immobilization Factor (SIF) and reported 100% immobilization of spermatozoa by SIF isolated from *S. aureus* in their molecular research on human sperm interaction with *S. aureus*.

Ejaculate analysis according to WHO [32] criteria included leucocyte analysis, may indicate persistent inflammatory activity, from this report, only 45 (27.8%) had WBC $\geq 1 \times 10^6$/ml. In many reports, transiently decreased sperm counts and forward motility are observed by many scholars [33–35].

This study reports mean sperm concentration of 18.83×10^6/ml, with 14.2% being azoospermic and 52.5% oligozoospermic, mean sperm concentration 10^7/ml. and normozoospermic sperm concentration of 41×10^6/ml. In all, sperm density, motility, and morphology reports were consistent with the reports of Weidner et al. [35] and Ludwig and Haslberger [36].

In conclusion, this study tend to align with the opinion that bacterial contamination of semen affects sperm quality and its concomitant effects were more closely associated with ologozoospermia and hence infertility. We have tried to statically present the report to portray the importance of certain variables studied so that a near adequate interpretative meaning could be adduced to semen analysis test request and results. Hence we report that Gram Negative organisms' presence in semen may affect the quality more than the Gram positive species, their presence affects non-progressive motility followed by sluggish progressive motility applying SPSS attribute of importance, while in Gram positive organisms, non-progressive motility was followed by higher abnormal morphology and cells with abnormal morphology are prone to apoptosis [22,24], hence immotility. However, the overall lower mean sperm concentration was observed among the Gram positive category (Fig. 7) as against the overall mean sperm concentration of the Gram negative.

Also, we are able to demonstrate that extremely low oligozoospermia and azoospermia may be associated with other fertility impediments, not necessarily presence of bacteria. There is need for proper interpretation of results of semen analysis, as demonstrated statistically in this study. More studies on strict anaerobic bacteria, virus and molecular studies may be necessary to further add meaning to peer-reports so far. Reports of improved semen quality after antibacterial treatments of both symptomatic and asymptomatic patients with facultative bacterial contamination were practical demonstrations that presence of certain bacteria affects semen quality [37,38] (result not included here) but the mechanisms are not always apparent.

Authors contributions

CAE and AO originated the study; CAE and OE designed the protocol. CAE: the principal investigator and originator of the study sampling and analysis (the manuscript is an extract from a larger study). BAI: took part in spermiogram and general analysis data. VNE: she took part in isolation, characterization of bacteria and antibiotic profile of the study. OE: he took part in recruiting the patients using the inclusion criteria and analysis of results. AO: overall supervisor of the project helped in data analysis and proof read the manuscript.

Conflict of interest

There is no conflict of interest.

References

[1] Emokpae MA, Mohammed AZ, Omale-Itodo A. Hormonal abnormalities in azoospermic men in Kano, Northern Nigeria. Ind J Med Res 2006;(124):299–304.

[2] Pellati D, Mylonakis I, Bertoloni G, Fiore C, Andrisani A, Ambrosini G, et al. Genital tract infections and infertility. Eur J Obstet Gynecol Reprod Biol 2008;140:3–11.

[3] Kurpisz MK, editor. New approach to male infertility: forum introductions. Reprod Biol Endocrinol 2004;2:8.

[4] Okonofua F, Menakaya U, Onemu SO, Omo-Aghoja LO, Bergstrom S. A case–control study of risk factors of male infertility in Nigeria. Asia J Androl 2005;(4):351–61.

[5] Bar-chama N, Fisch H. Infection and pyospermia in male infertility. World J Urol 1993;11(2):76–81.

[6] Imade GE, Towobola OA, Sagay AS, Otubu JA. Sexually transmitted disease and medico-social factors associated with male infertility in Nigeria. Arch AIDs Res 1993;7(3–4):245–52.

[7] Sikka SC. Role of oxidative stress and antioxidant in andrology and assisted reproductive technology. J Androl 2004;25(1):5–19.

[8] Moretti E, Capitani S, Figura N, Pammoli A, Federico MG, Giannerini V, et al. Presence of bacteria species in semen and sperm quality. J Assist Reprod Genet 2009;26:47–56.

[9] Gerais AS, Rushwan H. Infertility in Africa. Popul Sic 1992;(12):25–46.

[10] Onemu SO, Ibeh IN. Studied on the significance of positive bacterial semen cultures in male fertility in Nigeria. J Fertil Woman's Med 2001;46(4):210–4.

[11] Diemer T, Huwe P, Michelmann HW, Mayer F, Schiefer HG. Influence of autogenous leucocytes and *Escherichia coli* on sperm motility parameters *in vitro*. Andrologia 2003;35(2):100–5.

[12] Diemer T, Huwe P, Michelmann HW, Mayer F, Schiefer HG, Weidner W. *Escherichia coli*-induced alterations of human Spermatozoa: an electron microscopy analysis. Int J Androl 2000;23:178–86.

[13] Mehta RH, Sridhar H, Vijay Kumar BR, Anand Kumar TC. High incidence of oligozoospermia and teratozoospermia in human semen infected with the aerobic bacterium *Streptococcus faecalis*. RBM Online 2002;5:17–21.

[14] Qiang H, Jiang MS, Lin JY, He WM. Influence of *enterococci* on human sperm membrane *in vitro*. Asian J Androl 2007;9:77–81.

[15] Mogra NN, Dhruva AA, Kothari LK. Non-specific seminar track infection and male infertility: a bacteriological study. J Postgrad Med 1981;27(2):99–104.

[16] Ombelet W, Bosmans E, Janseen M, Cox A, Vlassclaer J, Gyselaers W, et al. Semen parameters in a fertile versus sub-fertile population: a need for change in the interpretation of semen testing. Hum Reprod 1997;12:987–93, http://dx.doi.org/10.1093/humrep/12.5.987.

[17] World Health Organization. WHO laboratory manual for the examination of human semen and semen–cervical mucus interaction. 4th ed. Cambridge University Press; 1999. p. 1–138. ISBN 0521645999.

[18] Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge University Press; 2000. p. 130–2 [chapter 7.5].

[19] Shaban SF. Male infertility overview: assessment, diagnosis and treatment. Chapel Hill, NC: University of North Carolina School of Medicine Press; 2007. p. 1–20.

[20] Campana A, de Agostini A, Bischof P, Tawfik E, Mastrorilli A. Evaluation of infertility. Hum Reprod Update 1995;1(6):586–606.

[21] Comhaire F, Vermeulen L. Human semen analysis. Hum Reprod Update 1995;1(4):343–62.

[22] Villegas V, Schulz M, Soto L, Sanchez R. Bacteria induce expression of apoptosis in human spermatozoa. Apoptosis 2005;10:105–10, doi:1007/s10495-005-6065-8.

[23] Fanibunda SE, Velhal SM, Raghavan VP, Bandivdekar AH. CD4 independent binding of HIV gp120 to mannose receptor on human spermatozoa. J Acquir Immune Defic Syndr 2008;48:389–97, http://dx.doi.org/10.1097/QAI.0b013e318179a0fb.

[24] Grassme H, Jendrossek V, Gulbins E. Molecular mechanisms of bacteria induced apoptosis. Apoptosis 2001;6:441–5, http://dx.doi.org/10.1023/A:1012485506972.

[25] Almond GW, Poolperm P. Semen contamination and choosing antibiotics. In: Proceedings of the North Carolina healthy hogs seminar. 1996. p. 17–20. www.ncsu.edu/project/swine_extension/healthyhogs/book1996/book96_5.htm.

[26] Diemer T, Weidner W, Michelmann HW, Schiefer H, Rovan E-G, Mayer F. Influence of *Escherichia coli* on motility parameters of human spermatozoa *in vitro*. Int J Androl 1996;19(5):271–7.

[27] Weidner W. *Escherichia coli*-induced alterations of human Spermatozoa: an electron microscopy analysis. Int J Androl 2000;23:178–86. http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=2120839& dopt=Abstract.

[28] Naber KG, Weidner W. Chronic prostatitis – an infectious disease? J Antimicrob Chemother 2000;46:157–61. http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10933636& dopt=Abstract.

[29] Huwe P, Diemer T, Ludwig M, Liu J, Schiefer HG, Weidner W. Influence of different uropathogenic microorganisms on human sperm motility parameters in an *in vitro* experiment. Andrologia 1998;30(1):55–9.

[30] Liu JH, Li HY, Cao ZG, Duan YF, Li Y, Ye ZQ. Influence of several uropathogenic microorganisms on human sperm motility parameters *in vitro*. Asian J Androl 2002;4(3):179–82.

[31] Gupta S, Prabha V. Human sperm interaction with *S. aureus*: a molecular approach. J Pathog 2012:1–7, doi:1155/2012/816536.

[32] World Health Organisation. WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. 3rd edn Cambridge: Cambridge University Press; 1992.

[33] Berger RE, Alexander RE, Harnisch JP, Paulsen CA, Monda GD, Ansell J, et al. Etiology, manifestations and therapy of acute epididymitis: prospective study of 50 cases. J Urol 1979;1121:750–4. http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=379366& dopt=Abstract.

[34] NGME (National Guideline for the Management of Epididymoorchitis). Clinical Effectiveness Group (Association of Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases). Sex Transm Infect 1999;75(Suppl. 1):S51–3.

[35] Weidner W, Garbe C, Weissbach L, Harbrecht J, Kleinschmidt K, Schiefer HG, et al. Initial therapy of acute unilateral epididymitis using ofloxacin. II. Andrological findings. Urologe A 1990;29:277–80 [in German].

[36] Ludwig G, Haselberger J. Epididymitis and fertility; treatment results in acute unspecific epididymitis. Fortschr Med 1977;95:397–9 [in German] http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=849851& dopt=Abstract.

[37] Skau PA, Folstad I. Do bacterial infections cause reduced ejaculate quality? A meta-analysis of antibiotic treatment of male infertility. Behav Ecol 2003;14(1):40–7.

[38] Orji I, Ezeifeka G, Amadi ES, Okafor F. Role of enriched media in bacterial isolation from semen and effect of microbial infection on semen quality: a study on 100 infertile men. Pak J Med Sci (Part-II) 2007;23(6):885–8.