

Association between Bacteriospermia and Abnormal Semen Characteristics

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ABSTRACT

Aims: To determine the prevalence of bacterial infection in semen of infertile males and the association between positive bacterial culture and form of male seminal infertility in Warri, Nigeria.

Methods: This study was carried out in a referral centre for infertility treatment in Warri, Nigeria. Semen samples were analyzed following the world health organization guidelines and bacteriological analysis of semen samples was done using standard techniques.

Results: The mean age of subjects studied was 37.3±1.0 years. 63(52.5%) out of 120 infertile males studied were found to have significant bacteriospermia. The commonest bacterial isolate recovered was *Staphylococcus aureus* 28(44.4%), this was followed by Coagulase negative *Staphylococcus* 13(21%), *Enterococcus faecalis* 12(19%), *Escherichia coli* 7(11.1%) while the least was *Proteus mirabilis* 3(4.8%). There was a significant association between leukocytospermia and a positive bacterial culture ($P<0.001$). *Staphylococcus aureus* infection was higher among infertile males with Oligozoospermia ($P=0.01$). *Enterococcus faecalis* were commonly isolated from teratozoospermic infertile males ($P<0.001$). *Escherichia coli* were more isolated from Asthenozoospermic males but this was not significantly different from the other forms of seminal infertility.

Conclusions: The prevalence of bacteriospermia among infertile males was high; *Staphylococcus aureus* and *Enterococcus faecalis* infection of semen seemed to correlate with poor sperm count and abnormal sperm morphology respectively.

Keyword: Bacteriospermia, Leukocytospermia, Infertility, Oligozoospermia, Semen

INTRODUCTION

Bacterial infection of semen (Bacteriospermia) is now known worldwide to be a significant contributing (15-20%) cause of male infertility^{1,2}. Evidence is accumulating from studies, on the potential role played by microorganisms in undermining sperm quality which includes; reduction in sperm count, poor sperm motility and abnormal sperm morphology^{3,4}.

A microorganism that infects the semen usually originates from the urinary tract or is acquired during sexual contact with their female partners⁴. *Escherichia coli* have been widely incriminated in the western world as the most common etiologic agent of microbial semen infection among infertile males⁵. Studies have also found a significant difference in microorganisms isolated from oligozoospermic, teratozoospermic and Asthenozoospermic infertile males^{6,7}.

In view of known deleterious role played by the presence of Bacteria in semen and reports of variation in bacterial isolates with forms of male seminal infertility, this study thus aimed to determine the prevalence of bacterial infection in semen and their distribution among the various forms of male seminal infertility among infertile males in Warri, Nigeria.

MATERIALS AND METHODS

This cross-sectional descriptive study recruited its subjects from the assisted reproduction technique/ in vitro fertilization clinic of Lily hospitals limited, Warri, Delta state Nigeria in the period November 2011–October, 2012. This IVF centre is a major referral clinic for the treatment of fertility related disorders in Warri, Delta state, Nigeria and to other clinics in the Niger delta region. The study included One hundred and twenty (120) males who visited our centre with complaints of infertility. Verbal or written consent were received from subjects before inclusion in the study. Ethical approval for the study was received from the ethical committee of Lily hospitals limited before commencement of the study. Semen samples were collected by subjects by masturbation into sterile universal containers after 3-5 days of

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abstinence. Subjects were taught essential process in collection of semen that prevents contamination of the sample.

Semen analysis: Semen samples were analyzed following the world health organization guidelines⁸. Briefly, semen samples were diluted 1/20 with 10% formalin and read microscopically using the improved Neubauer counting chamber to determine the sperm count and Negrosin eosin stained semen samples were read microscopically to determine sperm morphology. Presence of leukocyte was observed for; significant leukocytospermia was defined as 10^6 peroxidase positive leukocyte/ml ejaculate.

Form of seminal infertility; Oligozoospermia (sperm count $<15 \times 10^6$ /ml, Teratozoospermia (normal forms of 4%), Asthenozoospermia (progressive motility $<32\%$ (a+b), and Azospermia (No sperm cells in ejaculate), was grouped based on the currently approved world health guidelines on reference values for human semen characteristics⁹. The WHO reference values at 5th percentile for semen characteristics puts normozoospermia at semen volume of 1.5ml, sperm count of 39 and 15 million per ejaculate and per ml respectively; vitality of 58% alive; progressive motility of 32% and morphologically normal forms (strict criteria) of 4%⁹.

Semen bacteriology: Bacteriological analysis of semen samples was done using standard techniques as described by Chessbrough¹⁰. Semen samples were cultured aseptically into blood, Mackonkey and chocolate agars at 37°C for 24 hrs. Emergent colonies were read and characterized using standard biochemical test¹⁰. Statistical analysis was carried out using SPSS 16. P value less than 0.5 were taken as a significant.

RESULT

Based on microscopically observed semen characteristics, the 120 infertile males studied were found to have different forms of seminal male infertility, these were grouped into six groups (a) Oligozoospermia (n= 50) 42% (b) Teratozoospermia (n=17) 14.2% (c) Asthenozoospermia (n=15) 12.5% (d) Oligozoospermia +Asthenozoospermia (n=20) 16.7% (e) Oligozoospermia + Teratozoospermia (n=10) 8.3% (f) Azospermia (n= 8) 6.7% Fig 1. Oligozoospermia was the commonest form of seminal male infertility with the least being Azospermia. Mean age of studied infertile males was 37.3 ± 1.0 years.

Out of the 120 subjects studied, 63(52.5%) of the infertile males studied were found to have positive bacterial culture. The commonest bacterial isolate recovered was *Staphylococcus aureus* 28(44.4%) while the least was *Proteus mirabilis* 3(4.8%) (Table 1). There was a significant association between leukocytospermia and a positive bacterial culture ($P < 0.001$).

The distribution of the bacterial isolates recovered from the semen of subjects with respect to form of seminal infertility is presented in table 2. *Staphylococcus aureus* was found to infect all forms of infertile males studied but had its highest prevalence in oligozoospermic males 16 (57%) with teratozoospermic and azospermic males having the lowest infection with *S. aureus* 1(3.6%) each. *Enterococcus faecalis* was significantly commonly isolated from teratozoospermic infertile males than from the other subjects ($P < 0.001$). *Enterococcus faecalis* was recovered from teratozoospermic infertile males 9(75%) and Oligospermic-teratozoospermic males 3(25%) table 2. *Escherichia coli* were more commonly recovered from infertile males with Asthenozoospermia 4(57.1%) but this was not significantly different (Table 2).

Fig 1: Prevalence of forms of seminal infertility among infertile males studied

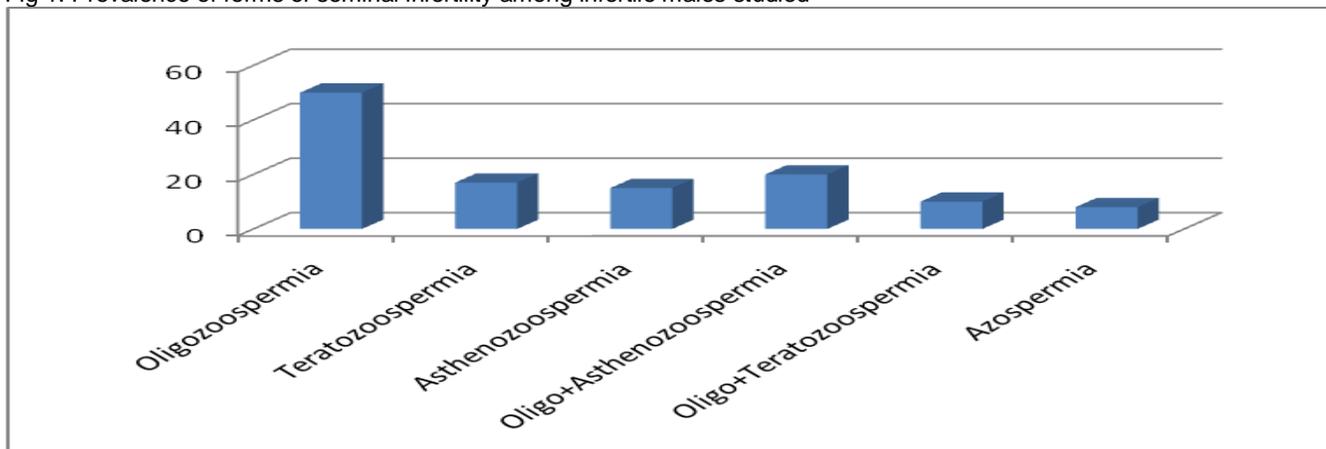


Table 1: Prevalence of bacterial organisms in semen of infertile men studied

Bacterial isolates	Number	%	Mean Sperm count
<i>Staphylococcus aureus</i>	28	44.4	$9.6 \times 10^6/l \pm 1.0$
Coagulase negative <i>Staphylococcus</i>	13	21.0	$10.5 \times 10^6/l \pm 0.7$
<i>Enterococcus faecalis</i>	12	19.0	$23 \times 10^6/l \pm 1.3$
<i>Escherichia coli</i>	7	11.1	$21 \times 10^6/l \pm 0.9$
<i>Proteus mirabilis</i>	3	4.8	$11 \times 10^6/l \pm 0.8$

P=0.01

Table 2: Prevalence of Bacterial infection in forms of male seminal infertility

Form of seminal infertility	<i>Staphylococcus aureus</i> (n=28)	<i>Enterococcus faecalis</i> (n=12)	Coagulase -ve <i>Staphylococcus</i> (n=13)	<i>Escherichia coli</i> (n=7)	<i>Proteus mirabilis</i> (n=3)
Oligozoospermia	16 (57.1)	-	5 (38.5)	1 (14.3)	1 (33.3)
Asthenozoospermia	3 (10.7)	-	1 (7.7)	4 (57.1)	-
Teratozoospermia	1 (3.6)	9 (75.0)	2 (15.3)	-	-
Oligozoospermia+ Asthenozoospermia	5 (17.9)	-	1 (7.7)	2 (29.0)	1 (33.3)
Oligozoospermia+ Teratozoospermia	2 (7.1)	3 (25.0)	1 (7.7)	-	1 (33.3)
Azospemia	1 (3.6)	-	3 (23.1)	-	-

DISCUSSION

This study reports the prevalence of bacteriospermia among infertile males in Warri, Delta state, Nigeria. 63 (52.5%) out of 120 infertile males studied had significant bacterial cultures. This high prevalence of bacteriospermia among infertile males in this study concurs with findings in previous studies^{11,12}. This study is a contribution to the body of knowledge incriminating bacteriospermia as a significant factor to male seminal infertility.

Staphylococcus aureus (44.4%) was the most common bacterial isolate recovered, with the least being *Proteus mirabilis* (4.8%). *Staphylococcus aureus* has been widely reported as the most common bacteria in semen among infertile males in Sub-Saharan Africa^{6,11,12}; this is quite at variance with the other parts of the world that has consistently incriminated *E. coli* and other uropathogenic gram positive bacteria as the most common bacterial agents of bacteriospermia^{13,14}.

In this study, *Enterococcus faecalis* was significantly higher among infertile males with Teratozoospermia and Oligozoospermia + Teratozoospermia. This finding concurs with reports of two previous studies^{15,16}. Mehta *et al*¹⁵ proved that *E. faecalis* was a potential cause of combined Teratozoospermia and Oligozoospermia among infertile males when they compared mean spermatozoa concentration, mean concentration of morphologically normal spermatozoa among semen infected with *Enterococcus faecalis*, micrococcus or alpha-hemolytic *Streptococci* and healthy uninfected subjects. Also, Moretti *et al*¹⁶ used transmission electron microscopy to study the ultrastructural effect of *E. faecalis* on spermatozoa; they concluded that *E.*

faecalis causes a compromisation of sperm quality in terms of sperm concentration and morphology with little or no effector effect on sperm motility.

Staphylococcus aureus was most commonly isolated from semen of infertile males with Oligozoospermia and Asthenozoospermia in this study. *S. aureus* is known to be capable of undermining semen characteristics, most especially sperm count, viability and motility¹⁵. Liu *et al*¹⁵ determined the effect of some uropathogenic organisms on human sperm mobility parameters, and concluded that *S. aureus* significantly decreased sperm motility and viability.

Escherichia coli were the most prevalent gram negative bacteria isolated from the semen of the subjects studied. Electron microscopy ultrastructural study¹⁸, proved that *E. coli* destroys the ultrastructure and motility of sperm cells by adhesion to spermatozoa thereby causing agglutination with the spermatozoa and impairment of acrosomal function. *Proteus mirabilis* was the other gram negative bacteria isolated from study subjects. The effect of *P. mirabilis* infection on semen parameters has not been well studied by researchers; it seems to be more associated with Oligozoospermia from our finding as it is commonly distributed amongst patients with Oligozoospermia and any other abnormal semen characteristics with co-existence of Oligozoospermia.

CONCLUSION

This study uncovers a high prevalence of bacterial infection in semen of infertile males in Warri, Nigeria. There was a significant correlation between poor sperm quality and bacteriospermia in the subjects studied. Bacteriospermia are usually asymptomatic,

as such bacterial culture of semen of infertile men should be made a routine and appropriate antibiotic therapy administered in case of positive bacterial culture.

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