Microbiologic Review of Seminal Fluids in a Nigerian Tertiary Health Centre

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Keywords: Male infertility; Microscopy; Culture; Semen

Abstract

Aims and Objectives: To determine the microbiologic pattern of seminal fluid in male infertility.

Subjects and Methods: A retrospective laboratory review of microbiologic analysis of 360 semen samples done in Benue State University Teaching Hospital, Makurdi, from January, 2013 to January, 2016.

Results: Fifty-one percent (N=184/360) of the semen samples was normospermic, 43% oligospermic (with 3% severe oligospermia) and 6% azospermic. Sixty per cent (N=216/360) of the semen samples contained sperm cells that were motile, 30% sluggish and 10% non-motile. Nine per cent of the semen samples contained dead sperms (necrospermic). Forty–two percent (N=150/360) of the semen samples contained sperm cells (normospermic). Pathogens were isolated in 47%(N=70/150) of the infected semen samples as follows; Staphylococcus aureus (53%), Staphylococcus saprophyticus (10%), Escherichia coli (11.4%), Klebsiella spp(7.1%), Streptococcus pneumoniae (4.4%), Pseudomonas aeruginosa (7.1%) and Candida spp (7.1%). Moxifloxacin was 85% effective, Levofloxacin (80%), Ceftazidime (80%), Ceftriaxone (80%), Ciprofloxacin (70%), Ofloxacin (68%) and Gentamicin (60%). While Penicillin (20%) and Tetracycline (10%) showed poor activity.

Conclusion: Regular semen microbiologic investigation is quite invaluable to male infertility intervention in poor resource countries.

Introduction

A semen analysis is vital tool to investigate male infertility [1]. It can also be used to validate a successful vasectomy as to absence of sperm.

Keywords: Male infertility; Microscopy; Culture; Semen

Subjects and Method

Study population

After obtaining ethical clearance from the ethics committee of Benue state university teaching hospital, we collected semen samples from three hundred and sixty (360) male...
subjects. The subjects were attending the Special Treatment Clinics/ Urology/Reproductive clinics of Benue State University Teaching Hospital from January, 2013 to January, 2016. Proper hygiene was ensured in the collection of the samples which was done in one of the private rooms in the laboratory.

Procedures

After liquefaction of the semen; the duration for the liquefaction was charted, the color noted and volume of the semen measured. The semen was mounted on a counting chamber (Improved Neubauer) where sperm count and sperm motility was read according to WHO standard [1].

Cultures

The following culture media plates were used; chocolate for 10% carbon dioxide incubation and MacConkey and Blood agar for aerobic incubation overnight at 37°C.

The 24 hour growth was examined for size, shape, elevation, hemolysis and other cultural characteristics. Gram staining was done and pure growth was subjected to biochemical testing according to standard [5].

Antibiogram

Antibiotic susceptibility of the isolates was tested by agar diffusion method on dry sensitivity testing agar (DSTA) using oxoid multidisc with standard antibiotics concentrations according to Clinical and Laboratory Standard Institute (CLSI), 2006 guidelines [12].

Analysis of data

The results were analyzed using SPSS 11.0 statistical software; chi-square ($\chi^2$) was used to compare association between proportions and p-values <0.05 were considered significant at 95.0% confidence level.

Results

A total of 360 seminal fluid samples were collected. The sperm count was as follows: Fifty-one percent of the sperm count was normospermic, 25% mildly oligospermic, 15% moderately oligospermic, 3% severely oligospermic and 6% azoospermic (Figure 1).

Forty per cent of the sperm cells were very motile, 20% of moderate progression, 30% sluggish and 10% non-motile (Figure 2). Viability test revealed 9% dead cells. Forty –two per cent (N=150/360) of the seminal fluid samples contained significant number of pus cells (≥2000 cells per ml). Pathogens were isolated in 47% (N=70/150) of this sample. The above criteria were distributed according to the percentage motility groups of sperm cells (Figure 3).

Total number of isolates was 70 as follows, S. aureus (53%), S. saprophyticus (10%), E. coli (11.4%), Klebsiella spp (7.1%), S.
Table 1 Microbial Isolates from semen of some subjects attending fertility clinics in Benue State University Teaching Hospital, January, 2013 to January, 2016.

| Isolates                  | Number | Percentage (%) |
|--------------------------|--------|----------------|
| Staphylococcus aureus    | 37/70  | 52.9           |
| Staphylococcus saprophyticus | 7/70  | 10             |
| Escherichia coli         | 8/70   | 11.4           |
| Klebsiella species       | 5/70   | 7.1            |
| Streptococcus pneumoniae | 3/70   | 4.4            |
| Pseudomonas aeruginosa   | 5/70   | 7.1            |
| Candida spp              | 5/70   | 7.1            |
| Total Isolates           | 70     | 19             |
| Sterile cultures (of samples with Pus Cells ≥ 2000/ml) | 80 | 22 |
| Total sterile cultures   | 290    | 81             |
| Total cultures           | 360    | 100            |

Moxifloxacin (85%), levofloxacin (80%), ceftazidime (80%), ceftriaxone (80%) ciprofloxacin (70%), ofloxacin (68%) and gentamicin (60%) are effectively active across the spectrum of bacteria tested. While penicillin (20%) and tetracycline (10%) are low in activity (Table 2).

Discussion

The study shows a 6% azoospermia, 43% oligospermia and 51% normospermic. Some studies in Nigeria showed the following results such as 8.8% [11] azoospermia in Ogun and 14.9% [13] in Osun both in South West Nigeria; oligospermic cases were reported as 51% [11], 46.5% [13], 45.3% in Ilorin, North-Central Nigeria [14], 60% [11] in Enugu, Eastern Nigeria are in synchrony with the present study. However, in Tunisia, North Africa was a 13% [15] lower in rate of oligospermia. Azoospermia categorically can be responsible for any couple’s infertility, since it is complete sterility, while oligospermia only decreases the chances of fertilization. This condition may result from autoimmune natural sperm agglutinating antibodies in the serum of sub fertile men [10].

Only 40% of the sperm cells were motile within one hour of ejaculation, ten per cent non motile, while remaining 50% were sluggish. Drake et al. [11] documented that bacterial infections could be responsible for decline in sperm motility. In early infection, there may be normal sperm count and sperm motility before the complication sets in. Role of bacterial infection may include change in the chemical milieu of the semen, inflammatory damage of the passage, destruction of the sperm cells and impede their motility. The consequence is therefore, reduced sperm count, teratospermia and asthenospermia. Escherichia coli have been found to decrease sperm motility and cause agglutination [11,16].

The male reproductive tract is essentially sterile except the lower urethra. Hence, greater than 2000 pus cells per ml of semen may be significant for diagnosis of infection [10]. The presence of certain organisms in semen or high number of the organisms is associated with infertility [17]. In the present study, 42% (N=150/360) of the semen samples had significant pus cells (≥2000 cells per ml) and pathogens were isolated in only 47% (N=70/150) of these samples. The fifty-three per cent (N=80/150) rate of sterile culture samples with significant pus cells is a pointer to reckless antibiotic usage in Nigeria [13, 18]. This ‘culture-negative semen’ challenge can be solved by the introduction of non-culture diagnostics.

The 19% (N=70/360) of the semen contained total number of isolates was as follows, S. aureus (53%), E. coli (11.4%), S. saprophyticus (10%), Klebsiella spp (7.1%), P. aeruginosa (7.1%), Candida spp (7.1%) and S. pneumoniae (4.4%) in descending order. The above data are comparable to studies by Orji et al. [16] and Olajubu et al. [11] which identified S. aureus as the commonest isolated bacteria with 37.1% and 51% respectively unlike a study [10] which found E. coli as the most prevalent with 70.4%. The isolation of S. aureus might have resulted from the skin flora of the couples. However, cross-infection might be a factor in some partners “trying outside marriage” [16]. Sex preferences may as well influence the nature and types of isolates in the semen [17,18]. For instance, anal sex may result in the transmission of bacteria which are normal flora of the intestine.

The most active antibiotics tested against the various types of bacterial isolates includes, Moxifloxacin (85%), Levofloxacin (80%), Ceftriaxone (80%). However, some other commonly used antibiotics, Penicillin (20%) and Tetracycline (10%) have been found to be crying orphans with low activity. Some usually active antibiotics, Ciprofloxacin (70%), Ofloxacin (68%) and Gentamicin (60%) have turned to
be moderately effective. The newer generation antibiotics are relatively expensive and are mostly given in hospitals. Therefore, they are not easily reached by the people.

Table 2 Antibiotic susceptibility pattern of the isolates from infected semen of the subjects

| Drugs  | Bacterial Isolates | S. aureus 37 (%) | S. saprophyticus 7 (%) | E. coli 8 (%) | Klebsiella spp. 5 (%) | P. aeruginosa 5 (%) | S. pneumoniae 3 (%) |
|--------|--------------------|------------------|------------------------|--------------|----------------------|-------------------|-------------------|
| AMP    | -                  | 10 (27)          | -                      | 3 (60)       | 2 (25)               | 1 (20)            | 1 (33)            |
| PEN    | 8 (22)             | 0 (0)            | 2 (25)                 | 1 (20)       | -                    | -                 | 1 (33)            |
| AMX    | -                  | -                | -                      | 1 (20)       | -                    | -                 | -                 |
| SXT    | 11 (30)            | 3 (43)           | 2 (25)                 | -            | -                    | 2 (66)            | -                 |
| TCN    | -                  | -                | 0 (0)                  | 1 (20)       | -                    | -                 | -                 |
| GENT   | 25 (68)            | 5 (71)           | 4 (50)                 | 3 (60)       | 2 (40)               | 2 (66)            | -                 |
| ERYTH  | 12 (32)            | 1 (14)           | 3 (38)                 | -            | 0 (0)                | -                 | -                 |
| MOXI   | 35 (95)            | 7 (100)          | -                      | 4 (80)       | -                    | -                 | -                 |
| CEFT   | 33 (89)            | -                | 6 (75)                 | -            | -                    | 3 (100)           | -                 |
| CIPRO  | -                  | 7 (100)          | -                      | 4 (80)       | 3 (60)               | -                 | -                 |
| OFL    | 30 (81)            | 6 (86)           | -                      | -            | 3 (60)               | -                 | -                 |
| LEV    | 34 (92)            | -                | 7 (88)                 | 4 (80)       | 3 (60)               | 3 (100)           | -                 |

*AMP=Ampicillin; PEN=Penicillin; AMX=Amoxicillin; MOXI=Moxifloxacin; TCN=Tetracycline; GENT=Gentamicin; ERYTH=Erythromycin; SXT=Trimethoprim-Sulphamethoxazole; CEFT=ceftriaxone; CEFT=ceftaxim; CIPRO=Ciprofloxacin; OFL=Ofloxacin; LEV=Levofoxacin; “S. aureus=Staphylococcus aureus; S. pneumoniae=Streptococcus pneumoniae; E.coli=Escherichia coli; spp=species

Male infertility has largely been attributed to nonspecific seminal tract infection [17,18]. Alausa and Osoba for infertility [9], discovered that 40% of oligosperma and azoospermia cases presenting to fertility clinics in Lagos Nigeria gave a history of two or more attack of urethritis. Fertility is jeopardized by these infections in several ways by decreasing sperm motility, changing the chemical milieu of the seminal fluid or illicit some inflammatory changes in seminal tract. Bacterial presence may cause testicular damage or the obstruction of the sperm ducts leading to low sperm count. Chronicity of some urinary tract infection is traceable to seminal infection by acting as a reservoir of infection [19].

In exception to infections, other conditions can be incriminated in male infertility. Such conditions like, exposure to heavy metals e.g. lead, zinc and arsenic. Others include; heat, chemical, X-ray exposure, smoking, nutritional deficiencies and use of alcohol, tobacco and caffeine. Also, many recreational and prescription drugs (e.g. nitro furans, cimetidine), some herbal medicines (such as St. John’s wort) and specific herbicides and pesticides have been found to be toxic to spermatogenesis. However, the correlations are difficult to develop, as supporting evidence is sketchy [9,18,19].

An automated system example, Computer-Assisted Sperm Analysis (CASA), would have given us a better analysis of sperm motility and other parameters may be a limitation to the study.

Interventions should be focused at prevention since surgical treatment of infertility and Assisted Reproductive Techniques (ARTs) are beyond the reach of many in Africa. Therefore, regular microbiologic screening and investigation for infertility is advised in this sub region.

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