EVALUATION OF THE RELATIONSHIP BETWEEN LEUCOCYTOSPERMIA AND BACTERIOSPERMIA IN SEXUALLY ACTIVE MALES IN BENIN CITY, NIGERIA.

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ABSTRACT
The presence of leucocytes and bacteria in spermatozoa is known as leucocytospermia and bacteriospermia respectively. In sexually active males, both conditions tend to reduce semen quality. This study was carried out to determine the presence and effects of leucocytes and bacteria in semen of sexually active males in Benin City, Nigeria. A total of 187 sexually-active men attending the University of Benin Teaching Hospital, Benin City, Nigeria volunteered to participate in the study. They were aged 19-33 years (mean = 23.7 ± 3.2). Seminal fluid samples were submitted by participants after 3 days abstinence from alcohol, drugs and sex. The samples were cultured on bacteriological media and identified by standard methods. Spermatozoa concentration, leucocytes density, motility and other parameters were performed microscopically using the World Health Organization guidelines. Antimicrobial sensitivity was done on isolates by the disc diffusion method. Out of 187 samples, 70 (37.4%) were positive for bacteriospermia. Staphylococcus aureus had the highest prevalence of 57.1%, this was followed by Escherichia coli (15.7%), Mycoplasma sp. (14.3%), Klebsiella spp. (7.1%) and Candida albicans (5.7%). Leucocyte counts ranged from 2.9-6.0 x 10^6/ml. There was an increase in bacteriospermia with increase in leucocyte counts. When leucocyte counts were ≥ 3 x 10^6/ml, the percentage of pathogenic microorganisms isolated and the progressive motility increased significantly (P < 0.001) for all the isolates except for Mycoplamsa spp. Antimicrobial sensitivity of all isolates (except Candida albicans) was higher to ciprofloxacin and pefloxacin. Although leucocytes is a poor marker of bacteriospermia, leucocytes density of ≥3x10^6/ml was a threshold marker for positive bacterial semen culture in this study. The presence of leucocytospermia and bacteriospermia were strongly associated with poor semen quality.

Key words: Leucocytes, Leucocytospermia, bacteriospermia, semen quality, sexually active males.

Introduction
Leucocytospermia may often be attributed to male genital tract infection (Wang et al., 1994). Elevated levels of leucocytes in semen have been associated with poor semen quality (Wolff et al., 1990; Yanushposky et al., 1990), or compromised sperm structural integrity (Aziz et al., 2004).

The prevalence of leucocytospermia could vary widely in different populations depending on factors such as sexual practices, the prevalence of sexually transmitted pathogens (Wolff, 1995) and from the percentage of the DNA of sexually transmitted pathogens detected in asymptomatic male infertility cases with poor semen quality (Benzold et al., 2007). Leucocytospermia has been reported as the most common cause of male infertility but the distribution, origin and role of leukocytes in semen is still considered controversial or having poor sensitivity and specificity (Onemu and Ibeh, 2001). Leucocytospermia is regarded as a poor marker of bacteriospermia because of the lack of correlation between positive semen cultures and sperm characteristics (Rodin et al., 2003). There is however, increasing evidence to suggest that there is a relationship between leukocytes and poor semen parameters (Yanushposky et al., 1990). The role of leucocytes in semen is thought to be associated with oxidative stress because leukocytes are major producers of reactive oxygen species (ROS) which damage spermatozoal function (Sharma et al., 2001; Li and Liu, 2006) or related to the functional competence of spermatozoa (Aitkin et al., 1994). Leucocytes concentration >2x10^6/ml has been suggested as the most likely to be unfavour-
able to the quality of semen profile and has been reported to contribute to the increasing rate of infertility amongst sexually active men (Kaleli et al., 2000). This study was therefore designed to evaluate the relationship between leucocytes and micro-organisms in semen of sexually active men in Benin City.

**PATIENTS AND METHODS**

**Study Population:** Seminal fluid samples from volunteer sexually-active young men aged 19–33 years (mean 23.7 ± 3.2) who gave oral consent after the aims and objective of the study was explained to them by the clinical physician who assisted in the study. Each participant (volunteer) was advised to abstain from passing semen for 72 h, wash hands and genitals with mild-toilet soap and dry skin with a sterile disposable towel provided.

**Sample collection and Examination:** Semen was collected by physical masturbation by each participant for delivery to laboratory within 1 h of collection. Each seminal fluid was serially diluted ten-fold in physiological saline (NaCl 0.15M). Inoculation of 0.1 ml of diluted semen was done on blood agar (Oxoid CM 55) heated blood agar (Oxoid CM 55), MacConkey agar (Oxoid CM7), Gonococcal agar (Lab 39), Sabouraud dextrose agar (Oxoid CM 41) and heart agar enriched with 30% serum and incorporated with 60 mg ceftriaxone. All inoculated culture media were incubated at 37°C for 24 -48 h. Cultures for *Mycoplasma* spp. were incubated for 96 h. Cultures with colony forming units (cfu) ≥ 10^3/ml of a single microbial type were picked for cultural, microscopical and biochemical characterization and identification (Cowan, 1974). Sensitivity tests were carried out on the bacterial isolates by the disc diffusion method (Baker and Breach, 1980). The seminal fluid samples were also examined for spermatozoa density and leukocyte count using standard methods (WHO, 1999).

Data generated were analyzed using one-way analysis of variance (ANOVA) to compare leukocyte count and spermatozoa density of semen with pathogenic micro-organisms to those without pathogens (Ogbeibu, 2005).

**RESULTS**

The examination of 187 semen samples from volunteer sexually active young men in Benin City, Nigeria resulted in the isolation of 70 (37.4 %) potentially pathogenic micro-organisms. *Staphylococcus aureus* was the most prevalent organism, followed by *Escherichia coli* 11 (15.7%), *Klebsiella* spp. 5 (7.1%), *Candida albicans* 4 (5.7%) and *Mycoplasma* spp. 10 (14.3%). The leucocytes count from seminal fluid samples with potentially pathogenic microorganism were significantly higher (p<0.001) than those without pathogens and mycoplasmal isolates. The progressive motility percent of spermatozoa from semen with pathogens was significantly lower (p<0.001) with exception of samples that yielded *Mycoplasma* species. The leucocytes count and spermatozoa concentration from seminal fluid samples with *Mycoplasma* isolates were not significantly different (P>0.05) from semen without pathogenic isolates.

The antimicrobial sensitivity test on bacterial isolates is shown in Table 2. The isolates were generally more susceptible to ciprofloxacin and pefloxacin. However, superior activity was shown against *Staphylococcus aureus* with amoxycillin-clavulanate while no activity was demonstrated against ampicillin and tetracycline respectively.

Fig. 1 shows the comparison of leukocytes and recovery of micro-organisms. The percentage of micro-organisms recovered was 1.4% from leukocytes count ≤ 2.9 x 10^6/ml while the percent of pathogenic isolates recovered increased significantly (P<0.001) to 7.1% with a leukocytes count of 3.0-3.9 x 10^6/ml. The highest percent (77.1%) of isolates were from semen samples with leukocytes count 4.0–5.9 x 10^6/ml.

Table 1: Comparison of bacterial isolates frequency, leucocytes count and spermatozoa concentration

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Percentage of isolate</th>
<th>Mean leucocyte count (x10^6/ml)</th>
<th>Mean spermatozoa concentration (x10^6/ml)</th>
<th>Mean progressive motility percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>57.1</td>
<td>4.9</td>
<td>17.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>15.7</td>
<td>5.4</td>
<td>33.3</td>
<td>38.3</td>
</tr>
<tr>
<td>Klebsiella spp. n = 5</td>
<td>7.1</td>
<td>4.7</td>
<td>12.7</td>
<td>25.0</td>
</tr>
<tr>
<td>Candida albicans n = 4</td>
<td>5.3</td>
<td>3.3</td>
<td>7.3</td>
<td>18.5</td>
</tr>
<tr>
<td>Mycoplasma spp. n = 10</td>
<td>14.3</td>
<td>0.6</td>
<td>76.8</td>
<td>63.0</td>
</tr>
<tr>
<td>Other semen samples n = 115</td>
<td>0.0</td>
<td>0.5</td>
<td>79.2</td>
<td>66.5</td>
</tr>
</tbody>
</table>

Each value represents the percentage of the number of isolates for a particular organism

**DISCUSSION**

The presence of pathogenic microorganisms (bacteriospermia) and elevated leukocyte count (leucocytospermia) may represent an important indicator in the deterioration of semen parameters. The examination of semen from 187 volunteer sexually-active young men, aged 19–33 (\( \bar{x} = 23.7 \pm 3.2 \)) years in Benin City, Nigeria resulted in the isolation of 70 (37.4%) microorganisms. *Staphylococcus aureus* 40 (57.10%) was the most frequently recovered micro-organisms. This observation is in agreement with earlier reports on semen microbial quality (Giamarello et al.,1984; Onemu and Ibeh,2001; Emokpae et al.,2005). Other isolated micro-organisms were *Escherichia coli* 11 (15.7%), *Mycoplasma* spp. 10 (14.3%), *Klebsiella* spp. 5 (7.1%). These micro-organisms have been reported to be associated with urogenital tract infections in both males and females (Brooks et al.,2004). *Candida albicans* 4 (5.7%) was the least frequently isolated micro-organisms, paradoxically, it was recovered from seminal fluid samples with the most severe signs of deterioration. This suggests that *Candida albicans* may represent a serious hazard to male fertility when semen is infected (Witkin and Toot,1983; Brooks et al.,2004). The role of *Mycoplasma* spp. in semen quality was not determined as had been previously reported (Kjærgaard et al.,1997; Keck et al.,1998).

The mean value for leucocytes count from semen with mycoplasmal isolates was significantly lower (\( P < 0.001 \)) when compared with semen samples with pathogenic isolates. Similarly, the mean spermatozoa density for these samples was also significantly higher (\( P < 0.001 \)). However, the mean leucocyte counts and spermatozoa densities were not significantly different (\( p > 0.05 \)) for semen samples without pathogenic micro-organisms. It may thus be suggested that *Mycoplasma* spp. in the semen samples may be opportunistic colonizers. The role of this micro-organism in semen may need further evaluation.

The bacterial isolates were generally more susceptible in the *in-vitro* test to ciprofloxacin and pefloxacin. The high antimicrobial activity of these agents in similar situations has been reported (Andriole, 1991; Childs,1991). The superior activity shown by amoxycillin-clavulanate against *Staphylococcus aureus* suggests that this antibiotic remains the most effective in managing *Staphylococcus aureus* infections as previously reported (Piroth et al.,2009). Lack of activity with ampicillin and tetracycline may have been largely influenced by the common misapplication and misadministration of these agents in the study community.

The number of pathogenic micro-organisms isolated with leucocytes count of >3.0–5.9 \( \times 10^6 \)/ml increased significantly (\( P < 0.001 \)). The highest frequency of pathogenic micro-organisms recovery was from leukocyte...
count range of $3.0\text{--}3.9 \times 10^6$/ml where as lower number of such micro-organisms was recovered from leukocytes counts $\geq 6 \times 10^6$/ml. This reinforces the observation that leukocyte count is not an adequate or sensitive marker of bacteriospermia.

Conclusion:
Leucocyte of $\geq 3 \times 10^6$/ml represents a threshold for positive bacterial semen culture or bacteriospermia in this population. The concurrent presence of leucocytospermia and bacteriospermia were strongly associated with decreased semen quality.

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REFERENCES


