GENITAL MYCOPLASMAS IN SEMEN SAMPLES OF MALES ATTENDING A TERTIARY CARE HOSPITAL IN NIGERIA: ANY ROLE IN SPERM COUNT REDUCTION?

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ABSTRACT
Semen samples from 54 married men attending the outpatient clinics for problems of infertility and routine semen analysis were examined for the presence of genital mycoplasmas. The mean age of the men was 36.1 years with a range of 25-55 years. Majority of the men 57.4% (31 of 54) were in their fourth decade of life (30-39 years). This age group also had the highest percentage 57.2% (8 of 14) of positive isolates of genital mycoplasmas on semen culture.

A total of 21 organisms obtained from 14 (26.0%) positive samples were isolated. Mycoplasma and Ureaplasma spp. separately isolated from the samples yielded frequencies of 1 (1.9%) and 6 (11.1%) respectively and the remaining 7 (13.0%) samples were infected with both organisms. A breakdown of the mycoplasma species include 5 (23.8%) M. hominis, 2 (9.5%) M. fermentans and 1 (4.8%) M. penetrans. Apart from one isolate of M. hominis other Mycoplasma species were found in association with Ureaplasma species.

Fifteen (71.4%) of the 21 isolates [8 (53.3%) ureaplasmas and 7 (46.7%) mycoplasmas] were isolated from samples with sperm counts less than 20 million/ml while the remaining 6 (21.6%) isolates [5 (83.3%) ureaplasmas and 1 (16.7) mycoplasma] were from samples with counts greater than 20 million/ml. This finding could indicate a possible influence of genital mycoplasmas especially mycoplasmas species on sperm count.

Keywords: Mycoplasma species; Ureaplasma species; Semen samples. (Accepted 13 July 2006)

INTRODUCTION
Infection in the reproductive tract of the male has long been recognized as a definite interference with sperm quality, transport and viability. Some microorganisms that cause genital tract infections like urethritis, epididymitis and prostatitis have also been implicated in seminal fluid infections. Such seminal fluid infections can thus be traced back to sexually transmitted pathogens like Neisseria gonorrhoeae, Chlamydia trachomatis, Ureaplasma urealyticum and Trichomonas vaginalis.

Mycoplasma infections have been known to cause reproductive problems in some mammals. This has therefore led to the suspicion that chronic asymptomatic genital tract colonization with mycoplasma may contribute to infertility. The role of genital mycoplasmas particularly Ureaplasma species in infertility has been a controversial issue for long. Despite this, some workers have been able to establish the potential role for this organism in male infertility. This is important in our environment where male infertility asthenospermia as major factors.

With the increasing reports of the possible role of Ureaplasma species in infertility, this investigation was carried out to establish the prevalence of genital mycoplasmas in semen samples and what possible effect they may have on sperm count.

MATERIALS AND METHODS
Patients: These comprise 54 men attending both the infertility clinic and the General Out-patients department of Nnamdi Azikiwe University teaching hospital, Nnewi, Nigeria. Each patient collected his semen sample by masturbation into sterile universal container and brought them to the laboratory within an hour of collection. All the men were married and there ages were obtained. Information on their sexual behavior (especially number of sexual partners) was not available for analysis.

Sperm Counts: The total sperm counts of each of the samples were determined using the automated semen...
Quality analyzer and bases on this; the subjects were split into 2 groups - those with counts below 20 million/ml and those with counts above 20 million/ml.

**Culture:** The culture media used for the cultivation of genital mycoplasma is the modification of Hayflick modified medium. The substrates glucose and arginine were not incorporated into the mycoplasma media and this is to minimize the proliferation of commensal organisms that may be present in the semen. Urea was however incorporated into the ureaplasma medium. The liquid-to-solid culture technique was used whereby the semen samples were first inoculated into mycoplasma and ureaplasma broths before being subcultured onto their corresponding agar. The broth media were incubated in air at 37°C and while the ureaplasma broths were incubated for 24 hours before being subcultured, the mycoplasma broths were incubated for up to 3 days before being subcultured. Incubation of the agar plates was in a candle jar at 37°C.

**Identification:** Using the dissecting microscope the ureaplasma agar plates were examined after 24/48 hours while the mycoplasma plates were examined as from the third day and daily for up to 10 days after which negative plates were discarded. Typical mycoplasma/ureaplasma colonies showing "fried-egg" appearance were noted and characterized using biochemical and serological analysis.

**Data Analysis:** The data were presented in tables and figures compared with percentiages and Chi square used for test for significance.

**RESULTS**

The mean age of the men was 36.1 years with a range of 25-55 years. Majority of the men 57.4% (31 of 54) were in their fourth decade of life (30-39 years). This age group also had the highest percentage 57.2% (8 of 14) of positive isolates of genital mycoplasmas on semen culture.

The overall prevalence rate of genital mycoplasmas from this study is 26%. Mycoplasma and Ureaplasma species separately isolated from the samples yielded frequencies of 3 (1.9%) and 6 (11.1%) respectively while the remaining 7 (13.0%) samples were infected with both organisms (Table 2).

The distribution of the genital mycoplasmas by semen count shows that 11 (32.3%) of the positive samples had sperm counts less than 20 million/ml and 3 (15.0%) had counts greater than 20 million/ml (p < 0.05). A breakdown of the 21 isolates into their various species is as shown in Table 3.

Ureaplasma species were more predominant

With 13 (61.9%) isolates and is followed by *M. hominis*, *M. Fermentans* and *M. penetrans* with 5 (23.8%), 2 (9.5%) and 1 (4.8%) prevalence rates respectively. It further shows the distribution of the Ureaplasma species and different species of mycoplasma according to the sperm counts of the patients. Of the 21 isolates, 15 (71.4%) were found in samples with counts less than 20 million/ml and 6 (28.6%) in samples with counts greater than 20 million/ml.

<table>
<thead>
<tr>
<th>Age</th>
<th><em>TSC&lt;20×10^6</em></th>
<th><em>TSC&lt;20×10^6</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>Positive Isolate</td>
<td>Negative Isolate</td>
</tr>
<tr>
<td>25-29</td>
<td>1 (9.1%)</td>
<td>5 (21.7%)</td>
</tr>
<tr>
<td>30-34</td>
<td>2 (18.2%)</td>
<td>5 (21.7%)</td>
</tr>
<tr>
<td>35-39</td>
<td>5 (45.5%)</td>
<td>8 (34.8%)</td>
</tr>
<tr>
<td>40-44</td>
<td>3 (27.3%)</td>
<td>2 (8.7%)</td>
</tr>
<tr>
<td>45-49</td>
<td>0 (0.0%)</td>
<td>2 (8.7%)</td>
</tr>
</tbody>
</table>

| Total | 1 (32.4%) | 23 (67.6%) |

\[ *TSC = \text{Total sperm count (cells/ml)} \]

**Table 2:** Overall prevalence of the genital mycoplasmas and their distribution according to the sperm counts. (N=54)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Total sperm count (cells/ml)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20×10^6</td>
<td>&gt;20×10^6</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma spp. alone</td>
<td>12 (2.9%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Ureaplasma spp. alone</td>
<td>5 (14.7%)</td>
<td>1 (5.0%)</td>
</tr>
<tr>
<td>Both species</td>
<td>5 (14.7%)</td>
<td>2 (10.0%)</td>
</tr>
<tr>
<td>Negative</td>
<td>23 (67.7%)</td>
<td>17 (83.0%)</td>
</tr>
</tbody>
</table>

| Total | 34 (63.0%) | 20 (37.0%) | 54 (100.0%) |

| X^2 = 2.383; df = 3; p < 0.05 |

**Table 3:** Breakdown of the total isolates into different species and their distribution according to the sperm count N=21.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. Isolated</th>
<th>Total sperm count (cells/ml)</th>
<th>&lt;20×10^6</th>
<th>&gt;20×10^6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma hominis</td>
<td>5 (23.8%)</td>
<td>4 (26.7%)</td>
<td>1 (16.7%)</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma fermentans</td>
<td>4 (5.0%)</td>
<td>2 (13.3%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma penetrans</td>
<td>1 (4.8%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Ureaplasma spp</td>
<td>13 (61.9%)</td>
<td>8 (53.3%)</td>
<td>5 (83.3%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21 (100.0%)</td>
<td>15 (71.4%)</td>
<td>6 (28.6%)</td>
<td></td>
</tr>
</tbody>
</table>

*More than one species was isolated from some
DISCUSSION

The ureaplasma isolates in this study are referred to as Ureaplasmata species. Recent studies have separated the only human ureaplasma, *U. urealyticum*, into 2 species - *U. parvum* and *U. urealyticum* [1]. Separation was only possible by molecular studies but these were not available at the time of this study. Waite's [2] reported that separating infections caused by the 2 species of Ureaplasma is not possible or necessary and so they are considered clinically and in the diagnostic laboratory as Ureaplasma species. The mycoplasma isolates were however characterized [3] and three separate species were seen in this study. Both the ureaplasma and mycoplasma isolates found in urethral tracts are generally referred to as 'genital mycoplasmas'.

Fourteen (26.0%) of the 54 semen samples were positive for genital mycoplasmas and a total of 21 organisms were isolated from them. The prevalence of the Ureaplasma and Mycoplasma species are 13 (27.8%) and 8 (14.8%) respectively. These values were comparable with those obtained by Hill [4] who reported 29.0% for *U. urealyticum* and 12.0% for *M. hominis*; and those of Andrade-Rocha [5] who reported 28.2% for *U. urealyticum* and 24.8% for *M. hominis* for one study group and 20.5% for *U. urealyticum* and 13.3% for *M. hominis* in another study group. In this investigation, mycoplasma species were found predominantly in association with ureaplasma species (87.5%). This finding supports that of Ladipo and Osoba [6] whose mycoplasma isolates from semen samples were found only in association with ureaplasmas. The significance of this finding is Uncertain. However, most of the genital mycoplasmas were isolated from patients with semen counts less than 20 million/ml and in their thirties. This is the age of maximal sexual activity and experimentation in men. History of the number of sexual partners of these men may have shade more light on the source of the infection but was not obtained in the course of the study.

Counts less than 20 million/ml and in their thirties. This is the age of maximal sexual activity and experimentation in men. History of the number of sexual partners of these men may have shade more light on the source of the infection but was not obtained in the course of the study.

Unlike the investigators that isolated only *M. hominis* from their studies, other Mycoplasma species apart from *M. hominis* were isolated in this study and they include *M. fermentans* and *M. penetrans*. Though with low prevalence, both species were isolated from semen samples with counts less than 20 million cells per ml. These 2 species of mycoplasmas are fastidious and have been reported to be difficult to isolate culturally [7]. This fact might have been responsible for their low isolation rates. Their role in semen bacteriology needs further investigation especially as several workers have isolated them from the urine and urethral samples of HIV positive patients [8]. *M. hominis* was more prevalent (23.8%) than the other two species and this is because they are less fastidious and easier to grow culturally. They have been reported to grow on ordinary blood agar and on some routine blood culture media [9].

The low isolation rate of genital mycoplasmas (26.0%) obtained in this study notwithstanding; they were isolated more from patients with counts less than 20 million per ml as against those with counts greater than 20 million per ml. Bacterial infections are frequently found in semen samples from asymptomatic fertile patients than those from fertile men [10]. In their own study, Kec et al [11] reported that infectious processes might lead to deterioration of spermatogenesis, impairment of sperm function and/or obstruction of the seminal tract. The finding in this investigation could therefore indicate a possible influence of genital mycoplasma on sperm count. However, the presence of these organisms in samples with counts greater than 20 million/ml may be as mere colonizers of the genital tract and thus may not be pathogenic as to have effect on the sperm count. Shepard and Lunceford [12] reported that not all serotypes of ureaplasmas are pathogenic while Kec et al [13] also reported that detection of bacteria in semen does not necessarily signify infection since bacteriospermia may represent contamination,
colonization or infection. It is significant to note that mycoplasma species were more prevalent in samples with counts less than 20 million/ml (oligospermia) than ureaplasma species. This indicates that mycoplasma species rather than ureaplasmas has greater influence on sperm count.

In conclusion, the prevalence of genital mycoplasmas in the study population was low. Their presence in oligospermic samples was significantly higher than in those with normal sperm count especially tory role for them in sperm reduction possible. It is therefore necessary to study the effect of these organisms on semen quality in order to establish a firm role for them in sperm reduction. For mycoplasma species thus making a contributory role for them in sperm reduction possible. It is therefore necessary to study the effect of these organisms on semen quality in order to establish a firm role for them in sperm reduction.

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1. Dalberg B. Asymptomatic Bacteriospermia: Cause of infertility in men. Urology. 1976; 8: 563-566. They were isolated more from patients with counts


