Common Bacterial Isolates Associated with Semen of Men Complaining of Infertility in University of Benin Teaching Hospital (U.B.T.H), Benin City, Nigeria

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Abstract: Fifty (50) seminal samples were studied to determine the microbial influence in male infertility as well as the qualitative and quantitative features of the semen. Among the three bacterial isolates isolated from the sample, *Staphylococcus aureus* 7 (77.8%) was found to be the most predominant isolate, other isolates were *Escherichia coli* 1 (11.1%) and *Citrobacter* spp. In relation to semen concentration *Staphylococcus aureus* was observed to be most frequently distributed among the characterized semen. The recovery frequency of organisms associated with the semen types revealed that 2(40%) of organisms were recovered of azoospermia, 6 (20.68%) oligozoospermia and 1 (6.25%) normozoospermia. The motility of the semen was greatly influenced by the presence of the bacterial isolates. The motility of semen was recorded to be 20%, 10% and 45% for *Staphylococcus aureus*, *Escherichia coli* and *Citrobacter* sp. respectively, as against the normal semen motility of 50% or more. The presence and profound influence of microorganisms in semen is evidence that microorganisms played significant role in male infertility.

Key words: Common bacterial isolates • Nigeria

INTRODUCTION

Normal semen is composed of the fluids from the vas deferens, the seminal vesicles, the prostate gland and the mucous glands, especially the bulbourethral glands. The major bulk of the semen is seminal vesicle fluid (about 60%), which is the last to be ejaculated and serves to wash the sperm out of the ejaculatory duct and urethra [1].

The primary reproductive organs or gonads of the males are the testes, which produce spermatozoa and also secrete the male sex hormone testosterone. In addition, there are the accessory reproductive ducts and secretory glands (seminal vesicles, prostate gland and bulbourethral glands), which are involved in the secretion of seminal fluid. A few weeks before birth, the two developing testes pass out of the abdominal cavity into the scrotal sac [2]. Failure of the testes to descend into the scrotum (cryptorchidism) may result in infertility, because the testes will not develop properly and production of spermatozoa (spermatogenesis) depends on a temperature of about 4°C below body temperature [2]. The scrotum can contract or relax to move the testes closer to or further away from the body so that this temperature is maintained. Spermatozoa are produced in the seminiferous tubules of the testes.

The seminiferous tubules contain many so-called germ cells (or spermatogenic cells), the vast majority of which are in various stages of division. A membrane surrounds each seminiferous tubule and only the outermost layer of spermatogenesis cells is in contact with this membrane, these are undifferentiated germ cells termed spermatogonia, which by dividing mitotically provides a continuous source of new germ cells. Some spermatogonia move away from the membrane and increase markedly in size. Each of these large cells, now termed a primary spermatocyte undergoes a meiotic division to form two secondary spermatocytes, each of which in turn divide into two spermatids. The latter ultimately being transformed into spermatozoa (sperm) [3].

The volume of semen and the sperm count decrease rapidly with repeated ejaculation. Even though, it takes only one sperm to fertilize the ovum, each milliliters of semen normally contains about 100 million sperms. Fifty percent of men with counts of 20 – 40 million cells/ml and
essentially all of those with count under 20 million cells/ml are sterile [4].

Sperm motility is essential for transport through the female tract and for fertilization. It is an expression of the viability and structural integrity of the cell [5].

Normal count is 20 x 10^6 spermatozoa/ml or more. Counts less than 20 x 10^6 cells/ml is known to be associated with male sterility. Normal spermatozoa measure 50-70 µm in length. Each consists of an oval-shaped head (with acrosomal cap), which measures 3-5 x 2-3 µm, a short middle piece and a long thin tail (at least 45 µm in length). At least 50% of spermatozoa should show normal morphology in normal semen [6].

Infertility constitutes a grave emotional and social problem in societies where great importance is attached to having children [7,8]. The contradictory scenario however, is that Sub-Saharan Africa characterized by high fertility also represents areas of highest prevalence of infertility. Estimates suggest that about 20 – 30 percent of couples in Africa experience primary and secondary infertility [9]. The most affected areas lie within the central African region referred to as the “infertility belt” of Africa [9,10].

Although Nigeria does not fall within the infertility belt, there are indications of high and rising prevalence levels [8]. In spite of the high prevalence of infertility, significant efforts have not been made at tackling the problem. Though in the recent past, the preoccupation of governments of developing countries and international agencies with the control of the high population growth rate, little efforts have been focused on preventing infertility and assisting individuals and couples that experience infertility [10-12].

Microbial infections have been associated with male infertility for many years; Neisseria gonorrhoea has been reported in the colonization of human sperm. Chlamydia trachomatis has also been reported to cause urethritis and epididymitis in men [13].

Presence of pathogenic microorganisms in semen, which may be related to a breach in the integrity of the blood-testes barrier, may provide early warning signals of impairment of male fertility [14].

This study was aimed at investigating and determining the influence of microorganisms in male infertility as well as to determine the qualitative and the quantitative features of the seminal fluid of man.

MATERIALS AND METHODS

Collection of samples: Seminal fluid specimens were collected from males attending the fertility clinic at the University of Benin Teaching Hospital (UBTH), Benin City. The samples were collected from patients who have had 3-7 days of sexual abstinence from intercourse, using the masturbation method. Upon collection, samples were transferred to the laboratory in a near as possible to body temperature by placing the container inside a plastic bag.

Examination of specimen

Appearance: Semen samples were examined immediately after liquefaction or within one hour of ejaculation. A normal sample has homogenous gray opalescent appearance. It may appear less opaque if the sperm concentration is very low, or brown when red blood cells are present.

Volume: The volume of the seminal fluid was measured by decanting the whole sample aseptically into a graduated centrifuge tube and the level was recorded in ml±0.1. The universal bottle was preserved for cultural examination and for further experimentation.

pH: The pH was determined by spreading a drop of the sample evenly onto the pH paper. After 30 seconds, the colour of the impregnated zone was compared with the calibrated strip.

Viscosity: The viscosity of the sample was determined with the aid of Pasteur pipette. A drop of semen was allowed to fall back to the sample and the length of the thread was observed. A normal sample leaves the pipette as small discrete drops while in abnormal cases, the drop forms a thread greater than 2 cm long.

Total Motility: Total motility of the samples was done by applying a drop of the sample onto a slide, covered with cover slip. The sample was then viewed under the microscope using x40 objective lens. The microscopic field was scanned systematically and the motility of each spermatozoon encountered was graded a, b, c and d that is, (a) Rapid progressive motility, (b) Slow or sluggish motility, (c) Non-progressive motility and (d) Immobility.

The number of spermatozoa in each category was counted with the aid of a laboratory counter. Usually, four to six fields were scanned to classify 100 successive spermatozoa. All motile spermatozoa with the ones that had their heads moving were recorded.

Count: Improved Neubauer Counting Chamber was used for the count. One twenty (1/20) dilution of semen was done with formol saline as diluents

\[ \text{Count} = \frac{N}{10^6} \]
Calculation:

Depth = 0.1 mm

Area = \( \frac{1}{3} \times \frac{1}{3} = \frac{1}{9} \times 5 = \frac{5}{9} \text{ mm}^2 \)

Volume = 0.1 mm \( \times \frac{5}{9} \text{ mm}^2 \)

= \( \frac{5}{90} \text{ mm}^3 \)

If N cell is in \( \frac{1}{50} \), N = \( n \times 50 \times 20 = 1000 \)

To convert to ml

1000 x 1000 = 106 N.

Dilution factor is 20.

Cultural method: Culture of seminal fluid samples (50) was done in aseptic condition, within 1 hour of collection the seminal fluid was cultured using Blood Agar, Chocolate Agar and MacConkey Agar at 37°C for 24 hours. The cultures were examined for growth; the isolation and identification of bacterial isolates were carried out in accordance with Bergey’s Manual of Determinative Bacteriology [15,16]. Significant cultures were tested with antibiotics to check for their sensitivity pattern using the antibiotic discs methods. The antibiotics used and their concentration per disc are as follows: Gentamycin (CN 10µg), Tetracycline (TE 10µg), Cloxacillin (OB 5µg), Augmentin (Aug 30µg), Amoxycillin (Amx 25µg), Chloramphenicol (C 30µg), Cotrimoxazole (SXT 25µg), Erythromycin (E 5µg).

RESULTS

Fifty (50) samples of semen collected from men attending the fertility clinic of the University of Benin Teaching Hospital (UBTH), Benin City, were examined routinely, for normal semen characteristics. Three genera of bacterial isolates were isolated and these include Staphylococcus, Escherichia and Citrobacter, with the highest counts recorded for genera Staphylococcus 7(77.8%), Escherichia 1(11.1%) and Citrobacter 1 (11.1%)

Table 1: Frequency of Bacterial Isolates

<table>
<thead>
<tr>
<th>Group</th>
<th>Bacterial Isolate</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Staphylococcus aureus</td>
<td>7</td>
<td>77.8%</td>
</tr>
<tr>
<td>B</td>
<td>Escherichia coli</td>
<td>1</td>
<td>11.1%</td>
</tr>
<tr>
<td>C</td>
<td>Citrobacter spp.</td>
<td>1</td>
<td>11.1%</td>
</tr>
</tbody>
</table>

Table 2: Distribution of Isolates Relative to Sperm Concentration

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Sperm concentration (x10⁶/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 19.9</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>7</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>1</td>
</tr>
</tbody>
</table>

Total 9 2 6 1

Table 3: Recovery Frequency of Organisms

<table>
<thead>
<tr>
<th>Classification</th>
<th>No. of samples</th>
<th>No. of organisms</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azosperma</td>
<td>5</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>Oligosperma</td>
<td>29</td>
<td>6</td>
<td>20.68%</td>
</tr>
<tr>
<td>Normozoosperma</td>
<td>16</td>
<td>1</td>
<td>6.25%</td>
</tr>
</tbody>
</table>

Table 4: Sperm Motility in Relation to Bacterial Isolates

<table>
<thead>
<tr>
<th>Identity of Isolate</th>
<th>No. of cases</th>
<th>Total</th>
<th>Progressive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>7</td>
<td>30.71%</td>
<td>20.00%</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>20.00%</td>
<td>10.00%</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td>1</td>
<td>55.00%</td>
<td>45.00%</td>
</tr>
<tr>
<td>Samples without significant growth</td>
<td>41</td>
<td>39.50%</td>
<td>29.14%</td>
</tr>
</tbody>
</table>

DISCUSSION

The incidence of infertility in Africa has assumed an alarming proportion as many couples experience this reproductive health problem [17]. This has posed significant inhibition to many reproductive health interventions such as maternal health care, sexually transmitted diseases and HIV/AIDS, as well as created social disharmony within the social structure such as marital instability, infidelity, divorce, emotional and mental stress, violence, denial, stigma and discrimination. Infertility treatment has remained outside the scope of many public health polices in Sub-Saharan Africa [8]. Public health policy makers appear to have neglected the problem of infertility for other issues perceived to be more important such as fertility regulation and reproductive health issues. Similarly, CDC, [17], have observed, demographers have for long focused their studies on fertility behaviour rather than on infertility. This however, has dire consequences on the entire reproductive health intervention programme in African societies where childbirth is accorded high social prestige [18,7]. Non-specific seminal tract infection can be an important cause of male infertility. These infections may affect fertility in several ways by damaging sperm, hampering their motility, altering the chemical composition of the seminal fluid or by producing an inflammatory structure in the tract. Seminal infection could also be the cause of the chronicity of urinary tract infection by acting as the reservoir of infection [19].

In many cases, opportunistic microorganisms cause such classical infections of the urogenital tract as epididymitis and prostatitis as well as subclinical
reproductive tract infections. Some possible pathophysiological mechanisms that lead to the development of infertility are linked to the presence of microorganisms in the ejaculate [20]. Additionally, the identification of pathogenic microorganisms in a sterile semen culture may be of significance [21].

The male reproductive tract is essentially a sterile zone except the lower urethra, which may contain a few commensals of the skin. Microorganisms are commonly found in insignificant quantities in the semen of asymptomatic men [22]. If the presence of microorganisms in the semen is associated with infertility, it is likely that only certain organisms are involved or that the numbers of organisms must be high for an effect to be seen [23]. It was shown in the result the frequency of the bacterial, that is the genera, Staphylococcus, Escherichia and Citrobacter, this was in accordance the finding of Sanocka-macleiewska et al., [24]. Among the bacteria isolates, the highest count was recorded for genera Staphylococcus 7(77.8%). The presence of the microorganisms is an indication of microbial infection. Gomez et al., [13] reported that, microbial infections of the semen are major causes of male infertility. In the research, high percentage of the isolates was recovered from semen samples with poor semen data as well as the profound effect of microorganisms on the semen motility. Stephen et al., [5] reported that, the viability and structural integrity of the semen lies on its characteristic feature of mobility.

The negative influence of these microorganisms towards sperm reproductive potential has been revealed in cases of infection with Escherichia coli and Staphylococcus aureus amongst other microorganisms [24].

Studies carried out by Auroux et al., [25] indicated that it was probable that the presence of Escherichia coli in semen decreases sperm motility but that this depends on the sperm bacterial/semen ratio/ml.

A research carried out by Huwe et al., [26] revealed that a variety of pathogenic microorganisms, Candida albicans inclusive, exert significant inhibitory effect on spermatozoa motility. Bornman, [27] established that Ureaplasma urealyticum affect fertility and Mycoplasma hominis is known to cause tail abnormalities of spermatoza. They have been found in significant amount in the semen of some infertile men.

In a study carried out in Nigeria, Alausa and Osoba [28] discovered that 40 % of husbands attending fertility clinics in Lagos with oligospermia and azoospermia gave a history of two or more attack of urethritis. Exposure to environmental toxicants (e.g ozone) that disrupt sperm production or the function of reproductive hormones or sperm may increase the risk of male infertility [29]. Previous studies by Alausa and Osoba [28] in Africa provided evidence that male infertility disorders are traceable mainly to prior genital infection. Consequently, unexplained male infertility disorder has generally been attributed to chronic, non-specific genital infections, which are expected to improve with appropriate antimicrobial therapy [30].

Findings indicate that seminal fluids constitute an important medium for the spread of various infective agents and that genital infections by these infective agents; sexually and non-sexually transmitted may be responsible for a good percentage of infertility cases in Nigeria males [31].

It has been observed that the presence of pathogenic organisms may interfere with treatment of infertility involving the application of in-vitro fertilization (IVF) and intra-uterine insemination [32].

Occupational exposure to heavy metals e.g. lead, zinc and arsenic have been reported to impair spermatogenesis [33]. In addition, the effects of specific herbicides and pesticides have also been found to be toxic to spermatogenesis [34]. Smoking has also been associated with lower sperm concentrations; however, correlations are difficult to develop, as supporting evidence is sketchy.

Fertility is important to all societies. The inability to have children has traditionally been a source of pain, anxiety and shame. In Africa, the number of children a man has usually measures his wealth. Children are important as farm workers and as a source of support in old age for their parents [7,8,35].

Similarly, procreation, continuity, perpetuation through the progeny and the need for self-preservation form the most basic needs of every family life. Hence the birth of a child to the married couple is an event of great joy, often celebrated with pomp and pageantry.

Interventions at resolving the problem of infertility should be focused at prevention [36]. This is because most attempts aimed at the surgical treatment of infertility have achieved limited success [17]. Also, the cost of Assisted Reproductive Techniques (ARTs) is still very high and beyond the reach of many. This is especially so for most parts of Africa.

Ojobo [17] has profound some preventive measures for infertility, these include, sex education, public health and hygiene, control of sexually transmitted diseases, correction of nutritional deficiencies, early treatment of abnormal conditions, prevention of damage from trauma, heat, chemical and x-ray exposure. When marriages are
childless, improvement of obstetric practice and provision of accessible health services, avoidance of unnecessary operations or procedures and proper contraceptive counseling are also important measures.

REFERENCES