Study of microbial infection in Asthenozoospermia patients
Sasikumar S*a, D. Dakshayanib, A. Franklinb and Rajkumar Samuelc

aNova IVI Fertility Centre, R.A. Param, Chennai-600 028, India
bSri Lakshmi Narayana Institute of Medical Sciences, Puducherry-605 502, India
cHubert Enviro Care Systems (Pvt.) Ltd., Chennai-600 083, India

*Corresponding author: sasikumarsundararajan1@gmail.com; Phone: +91-9884763971

Abstract

The pathogenic infection is an important cause of male infertility. The etiological role of infection in male infertility has been paid more attention in recent years. Asthenozoospermia may play a major role in infectious process which leads to deterioration of spermatogenesis, sperm motility and its morphological changes. The results of our study showed that 40% of infertile men were positive to at least for one pathogen. Moreover, there was an important relation between the bacteriospermia and sperm impairment. The rate of non-motile sperm and morphologically abnormal sperm was higher in infertility cases. Bacteria were isolated and their effects on sperm court, motility and morphological defects were studied. From the above studies, it was clear that the dominant bacteria such as Escherichia coli (40%), Staphylococcus aureus (28%). Pseudomonas aeruginosa (14%), proteus mirabilis (8%) were responsible for sperm motility and morphological changes. Mycoplasma hominis were present in lowest population (4%), so it was not taken into the account. But this also caused sperm motility and morphological changes in spermatozoa. The infections were compared with sperm motility and identified that the predominant bacteria which causes Asthenozoospermia was Escherichia.

Keywords: asthenozoospermia, spermatogenesis, pathogenic infection

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Introduction

Male infertility refers to infertility in male. Male infertility is involved in a sexually paired couple’s inability to conceive in a significant number of cases. Approximately 15% of couples attempting their first pregnancy meet with failure. Most authorities define these patients as primarily infertile if they have been unable to achieve a pregnancy after one year of unprotected intercourse. Conception normally is achieved within twelve months in 80-85% of couples who use no contraceptive measures and persons presenting after this time should therefore be regarded as possible infertile and should be evaluated. Data available over the past twenty years reveal that in approximately 30% cases pathology is found in the man alone, and in another 20% both the man and woman are abnormal. Therefore, the male factor is at least partly responsible in about 50% on infertility couples.

In vitro fertilization (IVF) is a process by which egg cells are fertilized by sperm outside the womb, in vitro. IVF is a major treatment in infertility when other methods of Assisted Reproductive Technology (ART) have failed. The process involves hormonally controlling the ovulatory process, removing ova (egg) from the woman’s ovaries and letting sperm fertilize them in a fluid medium. The fertilized egg (zygote) is then transferred to the patient’s uterus with the intent to establish a successful pregnancy.
The first test tube baby Louise Brown, was born in 1978. IVF is assisted for both male and female infertility. In male infertility, where there is defect in sperm quality, and in such cases Intra-Cytoplasmic Sperm Injection (ICSI) may be used, where a sperm cell is injected directly into the egg cell. This is used when sperm have difficulty in penetrating the egg, and in these cases the partners or a donor’s sperm may be used. ICSI is also used when sperm numbers are very low. ICSI results in success rates equal to those of IVF fertilization.

The reproductive organs of the male comprise the testes, epididymis, vas deferens, efferent ducts, the accessory sex gland-prostate, seminal vesicles and the bulbo-urethral glands and the penis. The germ cells which originate in the testis undergo a series of transformations within the testis as well as within the rest of the reproductive tract to form spermatozoa capable of fertilizing an oocyte. This process that takes about 70-80 days. A sperm cell or spermatozoa (plural spermatozoa in Greek : sperm: semen and zoon = alive) is the haploid cell that is the male gamete. It is carried in fluid called semen and is capable of fertilizing an egg cell to form a zygote. The sperm cell consists of a head, a mid piece and a tail. The head contains the nucleus with densely coiled chromatin fibres surrounded anteriorly by an acrosome, which contains enzymes used for penetrating the female egg. The midpiece has a central filamentous core with many mitochondria spiralled around it, used for ATP production for the journey through the female cervix, uterus and uterine tubes. The tail or flagellum executes the lashing movements that propel the spermatoocyte.

Sperm cells are of two types-motile and non-motile sperm cells. The tail or flag motile sperm cells typically move via flagella and require water in order to swim toward the egg for fertilization. These cells cannot swim backwards due to the nature of their propulsion. Non-motile sperm cells called spermatid lack flagella and therefore cannot swim. Spermatids are produced in a spermatogonium. About 60-100 million spermatozoa are normally present in each ejaculate. These spermatozoa may not all be morphologically normal and more than half of the spermatozoa may exhibit morphological abnormalities. Anything over 20 million sperm per milliliter is considered normal. A high concentration of leucocytes in semen is an indication of an inflammatory reactions or infection of the accessory sex glands. Immature germ cells, spermatocytes and spermatids may also present in semen and their presence in large numbers reflects abnormalities of spermatogenesis. The seminal fluid sperm concentration even amongst normal fertile individuals shows wide variations. The following is the World Health Organisation (WHO) criteria for normal values of semen variable (WHO, 1992).

Table 1. Analysis of microscopic parameters of semen

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>≥ 20 ml</td>
</tr>
<tr>
<td>PH</td>
<td>7.2 – 8.5</td>
</tr>
<tr>
<td>Concentration</td>
<td>≥ 20 X 106</td>
</tr>
<tr>
<td>Motility</td>
<td>≥ 50%</td>
</tr>
<tr>
<td>WBC</td>
<td>≤ 1 x 106</td>
</tr>
<tr>
<td>Morphology</td>
<td>≥ 30%</td>
</tr>
</tbody>
</table>

Semen analysis is still today a fundamental stage of male infertility diagnosis. But a semen analysis with normal parameters does not assure male. Except the cases of azoospermia, if does not distinguish fertile men, several parameters and the normal values of semen like viscosity, morphology and appearance are noted. A specimen of
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semen is examined microscopically in the laboratory to determine the number of sperms as well as their size, shape and motility. Estimation of the reproductive tract and genital organs may necessary. The infertility status of male is primarily evaluated on the basis of semen analysis. Endocrine factors, obstructions of the male reproductive tract, varicocele, abnormalities of spermatogenesis and infections are some of the causes of semen abnormalities. Recent studies have also shown that micro deletions on the long arm of the Y-chromosome can also result in poor sperm numbers or even the absence of spermatozoa (Reijo et al., 1995).

Factors that cause male infertility due to defects in semen quality are: Oligospermia-low semen volume, Oligozoospermia-few spermatozoa in semen, Aspermia-complete lack of semen, Azoospermia-absence of sperm cells in semen, Teratospermia-sperm with abnormal morphology and Asthenozoospermia-reduced sperm motility. There are various combinations of these as well, e.g. Teratoasthenozoospermia, which is reduced sperm morphology and motility. Sperm motility (whether the sperm are moving well or not) can only be assessed when a semen analysis is performed in the laboratory. Sperm motility is the ability to move. Motility is graded from a to d, according to the World Health Organisation (WHO) manual criteria as follows, Grade a (active)-sperm are those which swim forward fast in straight like guided missiles, Grade b (Moderate)-sperm swim forward, but either in a curved or crooked line or slowly (slow linear or non-linear motility), Grade c (sluggish)-sperm move their tails, but did not move forward (local motility only) and Grade d-immotile. Sperm do not move at all sperm of grade c to d are considered poor. Male accessory gland infections are controversially discussed as a cause of alteration in sperm motility, morphology (Menkveld and Kruger, 1998) and functional biochemistry (Zolata et al., 1998) that may be associated with male infertility (Comhaire et al., 1980; Purvis and Christiansen, 1993). A large series of infertile patients (n = 1000 couples), potentially pathogenic microorganism were identified in genital secretions of the majority of couples. In semen specimen, *Mycoplasmas* were found in 18-12%, potentially pathogenic aerobic bacteria in 50 and 31%, additionally commercial aerobes in 38 and 25% respectively. The microbial pattern showed great variability with polymicrobial growth most common. In endocervical material, Herpes Simplex Virus (HSV) was identified in 4.5%.

The pathogenic bacteria in the ejaculates can induce a defect in semen parameters, such as reduce sperm count, poor morphology and motility. It is already known that these parameters play a vital role in the fertility potential of a man. It is reported that presence of some bacteria in semen is indirect correlation with a decreased sperm count or percentage. The most commonly found organisms in infertility males was *Candida albicans*, *Candida tropicalis*, *Saccharomyces cerviseae* and bacteria like *Staphylococcus aureus*, *Enterococci*, *Klebsiella pneumonia*, *Pseudomonas aeriginosa*, *Ureaplasma Urealyticum*, *Mycoplasma hominis*, *Streptococci*, *Escherichia coli* and *Staphylococcus epidermis* (Golshnai et al., 2006). The Enterobacterium *Escherichia coli* appear to be the most important causative agent for urogenital infections that affect different sites of male reproductive tract. Two other infections associated with male infertility are *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Both of these are sexually transmitted infections. In men, *Chlamydia* usually has no symptoms so often a man will not ever realize he
has it until he starts to experience infertility problems. A few men will get a discharge from the penis.

**Asthenozoospermia**

It is the medical term for reduced sperm motility. It decreases the sperm quality and is therefore one of the major causes of infertility and reduced fertility in men. While the commonest cause of male infertility is a low sperm count (the Oligospermia), some men are infertile because of poor sperm motility. Normally at least 50% of sperm should be motile; and this should be good quality progressive motility. This condition is called asthenospermia (astheno = week). While many men with a low sperm count will also have poor sperm motility (the two often co-exist this is called, Oligoasthenospermia), some men will have a normal sperm count, but very poor sperm motility. In the presence study, it is proposed to isolate and identify the microbes from semen sample and to study the type of microbial infection from asthenozoospermia patients.

**Materials and Methods**

**Semen collection**

Semen samples were collected from 50 patients in the Billionth Fertility Centre by masturbation after a 3 days abstinence period, patients should not take any antibiotic since one week before collecting. Samples were collected in sterile plastic containers. The samples were delivered to the laboratory within 10 mins and kept in the incubator adjusted to body temperature.

**Labeling of the semen**

The name of the patients, period of abstinence date and time of collection should be recorded and the sample should be protected from extreme temperature (note less than 20° and more than 40°C) during transport to the laboratory.

**Liquification**

The collected sample was allowed to stand for 30 mins on a rocking plate.

**Semen analysis**

Collected samples were subjected to analysis of microscopic, physical and morphological characters of sperm.

**Analysis of physical characters of semen**

After the collection of samples, it is allowed to liquefy for 45 mins. Color of the sample is observed. Volume of total semen ejaculation is measured. The pH of semen was checked by use of the specific pH paper. These parameters were compared with the normal semen parameters to find the abnormalities in sperm.

**Microscopic observation of semen**

Semen and sperm suspensions were analyzed by microscopic visual assessment using a Meckler counting chamber of motile and immotile spermatozoa.

**Sperm count**

Number of sperms is important as too few can significantly decrease the chance of fertilization. A man is considered to have a low sperm count if it founds to have less than 20 million sperm per ml. A diagnosis of a low sperm count falls below 10 million/ml. Men that are found to have no sperm in their semen are said to be azoospermic.

**Sperm motility**

Sperm motility was evaluated soon after liquefaction, 5 µl of thoroughly mixed semen is placed on a Makler counting chamber, cover slip placed on it and the motility was observed as the average percentage of sperm moving in at least four random high power microscopic fields. The quality of motile sperm movement was classified based on the pattern displayed by the majority of
motile spermatozoa and ranged from 4 (excellent forward progression) to 0 (no movement). A forward progression of 4 is denoted to spermatozoa moving rapidly in a straight line. A forward progression of 3 is denoted to spermatozoa similarly moving linearly but a slower velocity. Sperm movement with a forward progression of exhibited angular displacement or to varying degrees while a progression of exhibited angular displacement or to varying degrees while a progression of 1 is denoted only tail motion without progression. Zero progression represented no movement at all.

**Calculation of motile and immotile sperm percentage**

The concentration is expressed in values per cubic mm counted motile. Immotile sperm in vertical 10 squares were subjected to the formula and the percentage of motility and total sperm count is found out.

\[
\text{Motility percentage} = \frac{\text{Number of motile sperm}}{\text{Number of sperms}} \times 100
\]

Total sperm count = number of motile sperm x volume

When the volume of the semen sample was taken out of count, other than the spermatozoa the presence of round cells, red blood cells and yeast contamination were noted when spermatozoa count was made.

**Microbiological investigation of semen sample**

**Semen culture test**

A routine microbiological investigation of semen was carried out from patients who come to Billroth Fertility Research Center, take treatment in fertility. The sample obtained from the infertility patient was streaked on the following appropriate media. The semen sample obtained from the infertile patient was streaked on the above medium in sterile manner. The plates were incubated at 37°C for 24-48 hrs. After incubation, the microbial colony morphology on the plates were observed and recorded.

**Identification of microorganism**

The purified bacterial cultures were identified based on gram staining, motility and other biochemical characteristics (Gibbon and Garden, 1974).

**Indole test**

Tryptophan is an essential amino acid it is oxidized by some bacteria using tryptophan’s resulting in the formation of insole, pyruvic acid and ammonia. Inoculating bacterium into trypton broth performs the indole test. The indole is produced during the reaction is detected by adding Kovac’s reagent (Para di-methyl amino benzaldehyde) which produce a cherry red reagent layer.

**Reagent layer**

**Trytophanase**

\[
\text{Tryptophan} \rightarrow \text{Indole + pyruvic acid + Ammonia} \quad \text{HCI Rosindol + H}_2\text{O}
\]

\[
\text{Indole + kovac’s reagent} \rightarrow \text{Cherry red compound (Butanol)}
\]

Tryptone broth was prepared, bacteria was inoculated and incubated. After incubation Kovac’s reagent was added and observed the result (Dutton, 1992).

**Methyl red test**

Glucose is a major oxidized by all enteric organisms for energy production. The end product will vary depending on the specific enzymatic pathways of the bacteria. In this test pH indicator methyl red detects the presence of large concentration of acidic end products. In pH range of 4.0, the methyl red indicator turns red which is the indicative of positive result. Yellow indicating the presence of alkali and it is a negative result (Gupta and Vohra, 1992).
**Vogesproskauer test**

Some microorganisms produce non-acidic or neutral end products such as 2, 3 butanediol and ethanol that result from glucose metabolism. Instead of acid unfortunately there is no satisfactory test for 2, 3 butanediol. However acetoine a precursor of 2, 3 butanediol can be easily detected with barritt’s reagent.

\[
\text{Glucose + Oxygen} \rightarrow \text{Acetic acid} \rightarrow 2,3 \text{butanediol} + \text{CO}_2 + \text{H}_2
\]

The oxidation will occur under alkaline condition in the presence of catalyst \(\alpha\)-naphthol and a guanidine group is present in the peptone of MRVP. Development of deep rose colour indicates the positive result. Absence of rose colour is negative result (Keskar, 1992). Each of the methyl vogesproskauver broth tubes was inoculated with appropriate bacterial cultures. Inoculated tubes were incubated at 37\(^\circ\)C for 24-48 hrs. After the incubation, Barritt’s reagents (A and B) were added and the results were observed.

**Citrate test**

In the absence of glucose or lactose some microorganism uses citrate as the carbon source which depends on the enzyme citrate permease. The enzyme citrate permease acts upon the substrate citrate which converts to oxaloacetic acid and acetate. These are then enzymatically converted to pyruvic acid and \(\text{CO}_2\). The medium is supplemented with sodium citrate as the carbon source. During the reacting the organism utilizes citrate and leaves sodium in the medium. The medium becomes alkaline as the \(\text{CO}_2\) combines with sodium and water, which forms sodium carbonate, an alkaline product. The sodium carbonate changes the bromothymol blue from green to deep Prussian blue colour (Meenakumari, 2006).

**Citrate**

- Citrate \(\rightarrow\) Oxaloacetic acid +
- Acetic acid
- Oxaloacetic acid \(\rightarrow\) Pyruvic acid +\(\text{CO}_2\)
- \(\text{CO}_2 + 2\text{Na}_2 + \text{H}_2\text{O} \rightarrow \text{Na}_2\text{CO}_3 + \text{sodium bicarbonate}\)
- Bromothymol blue + Sodium bicarbonate \(\rightarrow\) Green to Prussian blue colors

**Triple sugar-Iron test**

The fermentation reaction of sugar will help to distinguish Enterobacteriaceae from other gram-negative intestinal bacilli. TSI slant contains 1% each of lactose, sucrose and glucose in a concentration of 0.1%. The phenol red indicator is also incorporated in the medium to detect the carbohydrate fermentation.

**Sugar fermentation**

Acid butt, alkaline slant indicates the glucose fermentation but not lactose fermentation and sucrose fermentation. Alkaline butt and alkaline slant indicates none of glucose, sucrose or lactose fermentation. Acid butt, alkaline butt indicates the fermentation of lactose and sucrose.

**Gas production**

The production of gas can be identified by the presence of bubbles. The high production of gas breaks the agar media or pushes it upwards.

**\(\text{H}_2\text{S} \) production**

\(\text{H}_2\text{S} \) production can be identified by a blackening of the butt. This is due to the reaction of \(\text{H}_2\text{S} \) with ferrous ammonium sulphate supplemented in the medium, which forms ferrous sulphide.

**Urease test**

Urease is hydrolytic enzyme that attacks the nitrogen...
and carbon bond in amide compounds such as area and forms alkaline end product ammonia. The presence of urease is detectable when the organisms are grown in the Christensen’s urea agar containing the pH indicator phenol red. As the substance urea split into the product, the presence of ammonia creates an alkaline environment that causes the phenol red to turns to pink colour. This is the positive reaction. This failure to deep pink colour to develop is the indication of a negative reaction (Meenakumari, 2006). Christensen’s urea agar slant were prepared and inoculated with culture. Tubes were incubated at 37°C for 24-48 hrs. Following incubation, the results were observed.

**Catalase test**

During aerobic respiration, microorganisms produced hydrogen peroxide in some cases, an extremely toxic superoxide. Accumulation of these substances results in death of the organism unless they can be enzymatically degraded. The substance is produced when aerobes, facultative anaerobic and micro aerophiles use the aerobic respiratory pathway, in which oxygen is the final electron production. Organisms capable of producing catalase rapidly degrade hydrogen peroxide.

\[
\text{catalase} \\
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

In the case of catalase-negative organisms, the enzyme super oxide dismutase degrades the toxic superoxides. The inability of strict anaerobes to synthesize catalase, peroxidase, or superoxide dismutase absence of these enzymes, the toxic H₂O₂ cannot be degraded when these organism are cultivated in the presence of oxygen. Catalase production can be determined by adding the substrate H₂O₂ to an appropriately incubated trypicase soy agar slant culture. If catalase is present, the chemical reaction mentioned is indicated by bubbles of free oxygen gas. This is a positive catalase test the absence of bubble formation is a negative catalase test (Meenakumari, 2006).

In the center of the glass slide the given bacterial culture was smeared and a drop of 3% hydrogen peroxide was added to the smear and observes the result.

**Oxidase test**

Oxidase enzymes play a vital role in the operation of the electron transport system during aerobic respiration. Cytochrome oxidase catalyses the oxidation of reduced cytochrome by molecular oxygen resulting in the formation of H₂O₂ or H₂O. Aerobic bacteria as well as some facultative anaerobic and microaerophiles exhibit oxidase activity. The oxidase test aids in the differentiation among members of the enterobacteriaceae which are oxidase-negative. The ability of bacteria to produce cytochrome oxidase can be determined by the addition of the test reagent, p-amino di-methyl aniline oxalate on the colonies. The light pink reagent serves as an artificial substrate, donating electrons and thereby becoming oxidized to a blackish compound in the presence of the oxidase-free oxygen. Following the addition of test reagent, the development of pink, then maroon, and finally black colouration on the surface and represents a positive test. No colour change or a light pink colouratin on the colonies is indicative of the absence of oxidase activity that is negative result (Meenakumari, 2006). Take 24 hrs culture plates, add two or three drops of the p-amino dimethyl aniline oxalate to the surface of the growth of each test organism.

**Motility test**

Hanging drop preparation is useful for microscopic examination of lively microorganism, especially bacteria without staining them to their motility due to flagella.
A drop of culture broth was taken in a cover slip. A sterile cavity was pasted with a slip using petroleum jelly. In an inverted position, the slide was observed under the microscope.

Mannitol test: Mannitol medium was prepared, dispensed into the tubes, sterilized and allowed to cool in slanting position. The culture was inoculated and incubated at 37°C for 24 hrs. The fermentation reaction which indicates yellow colour, non-fermentation reaction indicates red colour (Johnson et al., 1995).

Results

In this study, 50 cases of male infertile semen samples were collected and then samples were cultured on appropriate medium and find out the microbial contamination or infection. One of the reasons responsible for their infertility is microbial infections. The physical characters of semen like sperm count, motility and WBC were analyzed (Table 1). The 50 semen samples were compared with normal range of sperm count, motility and morphology. Most of the samples were asthenozoospermia and some of them were oligoasthenoteratozoospermia or asthenoteratozoospermia. All the 50 semen specimens were plated on culture media like Blood agar, Nutrient agar and Mac Conkey agar. It showed bacterial growth on medium and then the isolated bacteria were identified by using biochemical tests (Table 2). Among bacterial infection Escherichia coli (40%) constituted the highest population, Staphylococcus aureus (28%) the second highest population. While Pseudomonas aeruginosa (14%), Proteus mirabilis (8%) and Mycoplasma hominis (4%) showed the lowest population.

Mycoplasma hominis is anaerobic. It was cultured on SP4 broth and identified by colour changes (orange to red). The bacterial infections were compared with sperm morphological changes (Table 3). E. coli was predominately found in morphological defects while Staphylococcus aureus found moderately. The infections were compared with sperm motility and identified that the predominant bacteria which causes Asthenozoospermia was Escherichia coli (Table 4). E. coli which constituted the highest population when compared with both motility and morphology. The bacterial growth, gram stain result and biochemical tests were shown in photos.

Discussion

The pathogenic infection is an important cause of male infertility. The etiological role of infection in male infertility has been paid attention in recent years. Asthenozoospermia may play a major role in infectious process which leads to deterioration of spermatogenesis, sperm motility and its morphological changes. The results of our study showed that 40% of infertile men were positive at least for one pathogen. Moreover, there was an important relation between the bacteriospermia and sperm impairment. The rate of non-motile sperm and morphologically abnormal sperm was higher in infertility cases. In my study, bacteria were isolated and their effects on sperm count, and motility and morphological defects were studied. From the above studies, it was clear that dominantly bacteria such a Escherichia coli (40%), Staphylococcus aureus (28%), Pseudomonas aeroginosa (14%), proteus mirabilis (8%) were responsible for sperm motility and morphological changes.

Mycoplasma hominis were present in lowest population (4%), so it was not taken into the account. But this also caused sperm motility and morphological changes in spermatozoa. Asthenozoospermia is the one of the important factor for male infertility. Generally, the risk of infertility increases by age but most of our patients were
Table 2. Semen parameter analysis-motility counting

<table>
<thead>
<tr>
<th>Age of Patient</th>
<th>Sperm Count million /cubic</th>
<th>Motility (%)</th>
<th>Total Motility (%)</th>
<th>Wbc</th>
<th>Impression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active</td>
<td>Moderate</td>
<td>Sluggish</td>
<td>Immotile</td>
<td>Active</td>
</tr>
<tr>
<td>36</td>
<td>37</td>
<td>11</td>
<td>28</td>
<td>37</td>
<td>24</td>
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<td>38</td>
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<td>49</td>
<td>00</td>
<td>38</td>
<td>29</td>
<td>33</td>
</tr>
</tbody>
</table>

Table 3. Biochemical test for bacterial isolation from semen

<table>
<thead>
<tr>
<th>Biochemical test</th>
<th>Staphyllococcus aureus</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
<th>Proteus mirabilis</th>
</tr>
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<tbody>
<tr>
<td>Gram staining</td>
<td>Gram Positive</td>
<td>Gram Negative</td>
<td>Gram Negative</td>
<td>Gram Negative</td>
</tr>
<tr>
<td>Shape</td>
<td>Cocci</td>
<td>Bacilli</td>
<td>Bacilli</td>
<td>Bacilli</td>
</tr>
<tr>
<td>Motility</td>
<td>Non-Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
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<tr>
<td>Catalase</td>
<td>Negative</td>
<td>Negative</td>
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<td>Negative</td>
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<tr>
<td>Coagulase</td>
<td>Positive</td>
<td>Negative</td>
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<td>Oxidase</td>
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<td>Indole</td>
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<td>Urease test</td>
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Table 4. Comparision between microbial culture and its morphology

<table>
<thead>
<tr>
<th>Age</th>
<th>Microorganisms</th>
<th>Morphology (%)</th>
<th>Normal</th>
<th>Head Defect</th>
<th>Neck Defect</th>
<th>Tail Defect</th>
<th>Cytoplasmic Droplet</th>
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<td>36</td>
<td>Escherichia Coli</td>
<td>16</td>
<td>58</td>
<td>16</td>
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<td>Very poor morphology</td>
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<tr>
<td>38</td>
<td>Staphylococcus aureus</td>
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<tr>
<td>32</td>
<td>Pseudomonas aeruginosa</td>
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Table 5. The semen microbial culture comparison with sperm motility

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<th>Age</th>
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<th>Motility (%)</th>
<th>Active (%)</th>
<th>Moderate (%)</th>
<th>Immotile (%)</th>
<th>Impression</th>
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young, so we should pay more attention to young men sexual health.

**Conclusion**

Asthenozoospermia caused by bacteria in human spermatozoa was studied. Microbial infection plays an important role in men infertility. Bacterial infection in semen has been paid attention as a major cause of male infertility. Microbiological investigation of semen sample of infertility men attending to fertility clinic and evaluated by standard culture plate method, standard semen analysis were performed according to WHO guidelines. There was a significant relation between the bacterial infection in sperm and the rate of non-motile and morphologically abnormal sperm.

The quality of sperm motility was significantly decreased in contaminated semen. The isolated bacteria were characterized by microscopic observation, culture type and also using their biochemistry. The predominate bacteria that caused sperm motility and morphological changes are *Escherichia coli* (40%) and *Staphylococcus aureus* (28%), *Pseudomonas aeruginosa* (14%), *Proteus mirabilis* (8%) and *Mycoplasma hominis* (4%). From the above study analyzed in patients was concluded that sperm motility (<50%) and morphological defect (<30%) was due to bacterial infection which possessed less chance for fertility potential and so they are advised to undergo IVF or ICSI treatment.

**References**


