*Full Length Research Paper*

# Semen Profile in Ile-Ife, Nigeria in Relation to New WHO Guidelines

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**Many studies have reported results of semen analysis, but emphasis has always been on sperm count without taking into consideration other parameters as defined by WHO. This study examines the pattern of semen analysis in male partners of infertile couples in the local community in the light of six parameters (sperm concentration, motility, morphology, viability, semen volume and pH) to provide information on semen quality which could be useful in adopting treatment options for infertile men in this environment. A prospective study was carried out using a structured questionnaire to obtain demographic information as well as medical/fertility history of 114 men who came to the Gynaecology clinics of  Obafemi Awolowo University Teaching Hospitals, Ile-Ife and Ilesa for male factor testing during the period of the study. Complete semen analysis was also carried out on samples obtained from these men according to WHO guidelines. 114 patients were seen in the course of the study. Sixty-nine percent of subjects were deficient in one or more of the semen quality parameters. 12.3% were azoospermic, 36.0% were oligospermic. A combination of Poor motility, abnormal morphology and oligospermia (Asthenospermic Teratospermic Oligospermic (ATO) syndrome) was seen in 2.6% of subjects. Many of the subjects (58.8%) had secondary infertility. Oligospermia was the most common of the disorders observed. The high incidence of abnormal semen quality in this locality becomes more obvious when all parameters, not just sperm count are taken into consideration. The role of infection should be investigated as suggested by the high incidence of secondary infertility.**

**Key words:** Semen Analysis, Infertility, Oligospermia, Infection

## INTRODUCTION

Semen Analysis remains the most feasible test for male fertility even though it is not a conclusive indicator of fertility vs. infertility. Semen guidelines such as those of the World Health Organization (WHO) have been established to determine semen quality limits below

which the chance of achieving pregnancy becomes increasingly less likely. Abnormal semen quality is a threat to male reproductive health with serious psychological and mental consequences. The etiology of abnormal semen and its diagnosis and evaluation in many developing countries are imprecise for many reasons. The method of semen collection, analysis and interpretation of results are not uniform. Thus it becomes difficult to appreciate the degree of normality in a

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particular sample of semen because of various definitions of normal and abnormal semen. This study essentially highlights the bio-characteristics and bio-variability of semen in Ile-Ife and compares the results with earlier studies in the community. It takes into consideration  six important criteria (Sperm concentration, motility, morphology, viability, volume and pH) as defined by WHO (2010) in grouping a man's semen as "normal" or " subnormal" and highlights the pattern of abnormal semen quality in the light of these guidelines. The occurrence of secondary infertility (inability to have more than one child) was also investigated. Many studies have shown that this is very common in Africa (Cates et al.,, 1985, Ekwere, 1995, Esimai et al.,, 2002) The incidence of infection, which is very closely related with secondary infertility was also examined.

## MATERIALS AND METHODS

A total of 114 men were involved in the study. They were made up of men who reported for routine male factor tests after their wives had presented for infertility in the Gynaecology clinics of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife and Ilesa. A structured questionnaire was used to obtain information on the demographic parameters, socioeconomic status and medical history of respondents. Respondents were provided with graduated wide mouth sterile specimen bottles. They were asked to produce semen into the bottles by masturbation after three (3) days of abstinence. The samples were delivered within 1 hour of collection. Complete analysis was carried out according to the WHO procedure for examination of human semen. Semen volume was read directly from the sample bottles (0.1ml accuracy). Semen pH was determined by spreading a drop of semen evenly onto a pH paper (range 5.5-10.0). The colour obtained after a few seconds was compared with the calibrated strip. Diluents for sperm concentration and motility as well as stains for sperm morphology and viability determinations were prepared according to WHO procedure (2010). Sperm concentration was determined using a Neubauer haemocytometer with a tali counter. A well- mixed, undiluted preparation of liquefied semen was examined on a glass slide under a coverslip to determine the appropriate dilution to use. A dilution of 1: 20 was made, after which a drop was transferred to the haemocytometer and covered with a slip. The Haemocytometer was then placed in a moist chamber for 15-20 minutes to allow the spermatozoa to settle. Spermatozoa were counted within selected grids of the haemocytometer chamber. Whole spermatozoa (with heads and tails) were counted. At least 200 spermatozoa were counted per replicate. The replicate counts were

compared to ensure accuracy. The concentration of spermatozoa/ ml was calculated and multiplied by the volume to obtain the total number of spermatozoa per ejaculate. For sperm motility, 10µl of well-mixed semen was placed on a glass slide and covered with a coverslip. This was examined at X400. The spermatozoa were assessed for the different motile categories (progressive motility, non-progressive motility and immotility). Percentage of motile sperm was calculated for all fields counted. Average percentage for each motility grade was recorded to the nearest whole number.

For determination of Sperm Morphology, a smear was made by placing a drop of semen on a clean slide and dragging the semen along the surface of the slide using the edge of another slide. The smear was air-dried and stained using Bryan-Leishman stain. It was then air-dried again, mounted on a phase-contrast microscope and characterized at X400. Abnormal forms were scored as a percentage of total spermatozoa counted.

Viability was determined by making a smear of semen-eosin-nigrosin. The smear was allowed to air-dry after which a differential count of  stained (dead) and unstained (viable) spermatozoa was carried out at X1000magnification.

Culture Media was prepared using cystine lactose electrolyte deficient (CLED) agar. 36g of the agar was dissolved in 1 litre of distilled water and autoclaved for 1hour. 1.5-2ml of the agar was then poured per sterile dish and left overnight to gel. The dishes were stored in the refridgerator. Semen samples were well mixed. A wire loop with holder was sterilized in a burner and used to innoculate and streak the plate. The plate was then incubated for 24 hours, after which it was inspected for significant growth in the primary innoculum. The plates with growth were separated for sensitivity test. Diagnostic sensitivity plates were prepared using DST agar (51g/ litre of distilled water). Growth was streaked on the plate and antibiotic discs were stuck on to the plates and incubated for 24 hours. Zones that are clear / growth free indicate sensitivity. The following antibiotics were used: Amoxycillin, Augmentin, Erythromycin, Streptomycin, Cotrimoxazole, Gentamycin, Tetracyclin, Chloram-phenicol and Nalidixic acid.

Semen samples were rated as normal or subnormal based on WHO criteria (2010) as follows

1. Sperm count≥15,000,000 spermatozoa/ml
2. Total (Progressive + non-progressive) Sperm motility ≥40% active spermatozoa
3. Sperm morphology≥40% normal forms
4. Sperm viability≥58% live spermatozoa.
5. Semen volume≥ 1.5ml
6. pH 7.2-8.0

Respondents who met all the above stated criteria were rated as having "normal" semen while those who fell short of one or more were said to have "sub-normal

**Table 1:** Fertility / Medical History of Respondents

| Characteristic | No of Respondents | % |
|---|---|---|
| **Duration of Infertility** (Yrs) | | |
| 1-5 | 77 | 67.5 |
| 6-10 | 25 | 21.9 |
| 11 or more | 12 | 10.5 |
| **Ever Impregnated a Woman?** | | |
| Yes | 67 | 58.8 |
| No | 47 | 41.2 |
| **Other Relevant Medical History** | 7 | 6.1 |
| Had Urinary Infection | 5 | 4.4 |
| Had Blood in Urine | 4 | 3.5 |
| Had Testicular Swelling/Pain | 3 | 2.6 |
| Had Mumps/ Genital injury | 2 | 1.8 |
| Had Genital surgery | 2 | 1.8 |
| Had Prostate Infection/Enlargement | 2 | 1.8 |
| Had Hernia Repair | | |

semen".

## RESULTS

Most of the respondents belong to the 30-39 age range. Duration of infertility was between 1-15 years Mean duration was 4.7± 4.6 years and most of the respondents (58.8%) were experiencing secondary infertility (Table 1). Medical history of the respondents showed that 12.3% had difficulty with intercourse while 26.3% have had sexually transmitted disease. Another 22% had histories of other conditions which placed them at risk for infertility such as mumps, hernia repair, genital surgery etc.

The seminal fluid profile shows that 59 subjects are normospermic, 41 are oligospermic while 14 have no spermatozoa in their ejaculate (azoospermia). 3 of the respondents suffered from a combination of oligospermia, low motility and abnormal morphology (Asthenospermic Tetratospermic Oligospermic (ATO) syndrome) (Table 2). The mean sperm count was 30 X $10^6$ ± 37.4 X $10^6$ In 28.9% of the respondents, motility was less than 40%. Also, 19.1% of them had poor sperm morphology (< 40% normal forms).

69.3% of subjects had staphylococcus aureus cultured in their semen.

35 of the respondents (30.7%) met all 6 WHO criteria (volume pH, sperm count, motility, morphology and viability) and were rated as having normal semen while others fell short of one or more of the criteria as shown (Table 2).

There was significant correlation of sperm count with motility, morphology and viability (Table 3).

## DISCUSSION

The peak age range of respondents in this study (30-39 years) is in agreement with previous studies (Onwudiegwu and Bako, 1993, Esimai et al., 2002) who found most men in infertile marriages to be in this age range .This shows that this is the age range at which most men get married and desire to have children. It also agrees with previous study of common occurrence of sexually transmitted disease among men of this age group which eventually results in infertility (Ekwere, 1995)

Duration of infertility among the respondents vary between 1-15 years, with the highest proportion between 1-5years (Table 1). It is reported that 70% of infertile couples in Africa sought medical intervention after failing to conceive for about 2½ years.

(Cates et al., 1995).The mean duration of infertility in this study is slightly longer (4.7±4.6years). This is probably due to the fact that most couples with infertility would have presented in some other places for treatment before being referred.

The incidence of subnormal semen parameters found in this study (69.3%) is much higher when compared with previous studies and this may be due to the fact that their definition of male infertility relied heavily on the sperm density. Research has shown that sperm density alone as a measure of male fertility is misleading and all other parameters such as motility, morphology, viability and volume must be considered (Onwudiegwu and Bako, 1993, WHO, 2010, Loto et al., 2004).

This study shows that 58.8% of the respondents have impregnated a woman at one time or the other. i.e they

**Table 2:** Semen Profile of Respondents

| Parameter | No of Respondents | % |
|---|---|---|
| **pH** | | |
| <7.2 | 2 | 1.8 |
| 7.2-8.0 | 107 | 93.9 |
| >8.0 | 5 | 4.4 |
| **Semen Volume** | | |
| <1.5ml | 5 | 4.4 |
| ≥1.5ml | 109 | |
| | | 95.6 |
| **Sperm Count** | | |
| Azoospermic | 14 | 12.3 |
| Oligospermic | 41 | 36.0 |
| Normospermic | 59 | 51.8 |
| (All other parameters measured excludes Azoospermic subjects) | | |
| Oligospermia + Infection | 35 | 30.4 |
| **Motility(% of Active Cells** | | |
| <40(Asthenospemic) | 24 | 28.9 |
| ≥40 | 76 | 71.1 |
| **Morphology (% Normal cells)** | | |
| <40% (Tetratospermic) | 13 | 19.1 |
| ≥40% | 87 | 78.9 |
| **Viability (% Live cells)** | | |
| <58% | 41 | 62.3 |
| ≥58% | 59 | 37.7 |
| **Overall Semen Status (Based on 6 parameters as presented)** | | |
| Normal | 35 | 30.7 |
| Sub-normal | 79 | 69.3 |
| ATO (Asthenospermic Tetratospermic Oligospermic) Syndrome | 3 | 2.6 |
| **Isolated Pathogens** | | |
| Staphylococcus aureus | 79 | 69.3 |
| E.Coli | 13 | 11.4 |
| Candida | 12 | 10.5 |
| Proteus | 6 | 5.3 |
| Pseudomonas aeroginosa | 4 | 3.5 |

had secondary infertility. This is similar to previous work (Esimai et al., 2002) which reports that African couples are much more likely to have difficulty conceiving after the first child than couples from other parts of the world. (29% in developed countries and 16-40% in other regions of the developing world (Cates et al, 1985). This was attributed to sexually transmitted infections (STIs) and post partum infections particularly in women. It has been demonstrated that the rate of promiscuity in men increases when their wives are pregnant or breastfeeding. This may account for increase in the rate of secondary infertility in men (Ekwere,1995. If STIs are not well treated they will retrograde into the testicles where they are likely to cause damage leading to post inflammatory obstruction or they may secrete toxins which alter the spermatozoal characteristics. This will then cause secondary infertility. Infection was very common (Table 1) in this study and was seen in 69.3% of the respondents. Almost all the infection was due to moderate to high growth of staphylococcus aureus .A previous study (Onwudiegwu and Bako, 1993) found infection in 45.1% of patients and most of this was due to the same pathogen. Infection is a big issue in infertility in this part of the world and numerous organisms have been implicated (Wolf, 1995). This was attributed to poor access to good health care and delivery, poor midwifery practices and a high incidence of complications that cannot be remedied (Larsen, 2000). It has been documented that sperm characteristics / quality change with time (Zorn et al, 2001). This may result from illnesses such as fever, testicular disease, malignancy, drug intake, stress, exposure to environmental pollutants, use of tobacco and surgical conditions involving the reproductive tract. It is not surprising that

**Table 3:** Correlation of various parameters

|  | Age | Duration of Infertility | Active Spermatozoa | Viable Spermatozoa | Normal Spermatozoa | Volume | Sperm Count |
|---|---|---|---|---|---|---|---|
| **Age** PC Sig | 1 | 0.274** 0.004 | -0.145 0.135 | -0.090 0.379 | 0.010 0.919 | -0.080 0.398 | 0.025 0.792 |
| **Duration of Infertility** PC Sig | 0.274** 0.004 | 1 | -0.274** 0.005 | -0.251* 0.014 | -0.223* 0.020 | -0.156 0.106 | -0.007 0.041 |
| **Active Sperm** PC Sig | -0.145 0.135 | -0.274 0.005 | 1 | 0 .687** 0.000 | 0.632** 0.000 | 0.176 0.070 | 0.255** 0.008 |
| **Viable Sperm** PC Sig | -0.090 0.379 | -0.251** 0.014 | 0.687** 0.000 | 1 | 0.520** 0.000 | -0.070 0.491 | 0.374** 0.000 |
| **Normal Sperm** PC Sig | 0.010 0.919 | 0.223* 0.020 | 0.632** 0.000 | 0.520** 0.000 | 1 | 0.188* 0.045 | 0.204* 0.032 |
| **Volume** PC Sig | -0.080 0.398 | -0.156 0.106 | 0.176 0.070 | -0.070 0.491 | 0.188* 0.045 | 1 | -0.130 0.173 |
| **Sperm Concentration** PC Sig | 0.025 0.792 | -0.007 0.941 | 0.255** 0.008 | 0.374** 0.000 | 0.204* 0.032 | -0.130 0.173 | 1 |

\* Correlation is significant at the 0.01 level
\*\* Correlation is significant at the 0.05 level
PC  Pearson Correlation
Sig  Level of Significance

22% of the respondents suffer from one or more of these conditions.

Duration of infertility was found to correlate negatively with motility, morphology and viability. Also, motility, morphology, viability and sperm concentration all correlate positively (Table 3).

Extremely acidic pH (<6.5) is found in agenesis or occlusion of the seminal vesicles while a high pH indicates infection. In this study, 95% of the semen samples fall within normal pH values (7.2-8.0) Ejaculate volume was also within normal range > 1.5ml) in 96% of subjects.

This study further supports declining sperm quality in our environment. The present reliance on sperm concentration and volume to assign normal or abnormal semen needs to further include other semen parameters in order to make statistical data on male infertility uniform and comparable worldwide and by so doing the proportion of men with abnormality will become obvious for further research.  The role of staphylococcus in male infertility and presence in normal and abnormal semen will require further research.

The proportion of men with more than one abnormality in the WHO criteria (69.3%) in this study is grossly high and worrisome and efforts to promote good health and care is desirable at the primary level of care in the health sector and this can be achieved through good nutrition, exercise and avoidance of drug abuse/ self medication.

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