Microbiology of Semen from Men with Fertility Problems in Imo State, Nigeria

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Abstract: This work is aimed at determining whether presence of microorganisms in seminal fluid might be the cause of male infertility.Three hundred and eighty-eight semen samples were submitted and analyzed microbially by routine semen analysis and culture techniques. Bacteriological examination of the semen samples revealed five bacterial isolates, including *Staphylococcus aureus* (39.2%), *Klebsiella sp.* (13.7%), *Escherichia coli* (9.8%), *Proteus mirabilis* (15.7%) and *Neisserria gonorrhoeae* (8.8%). The motility of sperm cells showed that 208 (53.6%) had 70 -100% actively motile cells, 116 (29.9%) had 50-60% actively motile cells and 64 (16.5%) had below 50% actively motile cells. Analysis of the duration of sperm motility showed that 20 (5.2%) had duration of 1- 10 hours, 276 (71.1%) had duration of 11 – 20 hours and 60 (15.5%) had duration of 21 – 24 hours. Analysis of the semen viscosity showed that 16 (4. 1 %) had highly viscous semen, 100 (25. 8%) had slightly viscous semen, 100 (25.8%) had watery semen and 172 (44. 3%) had moderately viscous semen. This study revealed that, infertility in men could be as a result of the presence of microorganisms in semen.

***Keywords*:** Semen, bacterial isolates, men, fertility problem, Imo State.

Introduction

Reproduction is the process by which living things produce offspring and this is what differentiates living things from non-living things. For this to be achieved in higher animals (humans) two gametes are involved, the male gamete (sperm) and the female gamete (secondary oocyte). These two gametes must meet in the female reproductive system to create a new individual. The inability of this to occur is referred to as infertility. This could be as a result of deficiencies on the part of either sex gametes. About 20 – 30% of couples in Africa experience primary and secondary infertility (Okonofuaa, 1999).

During sexual intercourse, the male ejaculates a whitish or grayish liquid from the urethra (tube in the penis) called semen. Each milliliter of semen contains 60-150million spermatozoa (Cheesbrough, 2002). This is used mainly for reproduction. The semen consists of the secretions of several glands of which 5% comes from testicles and epididymis, 45-80% comes from seminal vesicles, 13-33% comes from prostrate gland and 2-5% comes from bulbourethral and urethral glands (Carlson , *et al,*1992). The bulk of the semen is seminal vesicle fluid which is the last to be ejaculated and serves to wash the sperm out of the ejaculating duct and urethra (Guyton, 1992).

It takes over 70 days to develop spermatozoa (sperm) and it is produced solely in the testicle. Individual sperm is developed within the testicle from a cell called a spermatogonium.

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The spermatogonium divides to produce spermatocytes which subsequently develop into spermatid.Spermatid develops its familiar tail and the cell gradually acquires the ability to move by beating its tail. The spermatid finally develops into a mature spermatozoan. This process takes about 60days and the sperm takes a further 10 – 14 days to pass through the ducts of each testicle and its sperm maturing tube, the epididymis, before it can leave the body in the semen during ejaculation (Carlson,*et al*, 1992).

The average volume of semen produced during ejaculation is 2 – 5ml. Volumes consistently less than 1.4ml (hypospermia) or more than 5.5ml (hyperspermia) are probably abnormal. A lower volume may occur after very frequent ejaculation and higher volume after prolonged abstinence. The volume, sperm count, viability and motility of sperm determine how fertile a man is. These can be determined by so many factors of which infection of semen by micro-organisms is one of them. Microbial infections have been associated with male infertility for many years; *Neisserria gonorrhoeae* has been reported in the colonization of human sperm. *Chlamydia trachomatis* has also been reported to cause urethritis and epididymitis in men (Gomez *et al.*, 1979). According to some scientists, microorganisms can achieve this by producing some harmful toxins or by physically interfering with their motility. A study carried out by Huwe *et al.*, (1998) revealed that a variety of pathogenic microorganisms, *Candida albicans* inclusive, exert significant effect on spermatozoa motility. This work examined the microbiological quality and the qualitative features of the seminal fluid from men with fertility problem.

Materials and Methods

The study was carried out at Federal Medical Centre, Owerri and Imo State University Teaching Hospital, Orlu. Sterile transparent wide–mouthed, leak-proof specimen bottles were given to 388 men from 18years to above 46years to collect semen samples. Additional 30 semen samples were collected from fertile men attending these hospitals for routine check up. This was used as a control for this work. Samples were analyzed within 30 minutes of collection, by examining the physical appearance, measuring the volume, pH and checking of the viscosity of the semen as well as the motility of the sperms. Sperm count and culture of the semen were also carried out as described by Cheesbrough (2002).

Results

Table 1 shows the frequency of bacterial isolates for both non fertile and fertile subjects. Five bacterial isolates were revealed indicating *Staphylococcus aureus* as the most predominant organism for results obtained from subjects with fertility problem while non was isolated from subjects without fertility problem.

In Table 2, it is shownthat among the non fertile men, those that had sperm count below 40 million had 100% prevalence of infection while those that had sperm count above 40 million had lower prevalence of infection. Among the fertile men no infection was recorded and as such all the men had sperm count above 40million.This is to say that sperm count increases as infection decreases. This concords with a work carried out by Sanocka-Maciejewska *et al* (2005). In his work, it was revealed that there is a statistical significant deterioration in sperm concentration and infection. This conclusion was arrived at when it analyzed ejaculated samples of patients with genital tract infection.

Table 3 reveals that among the non fertile men, those that had extreme viscosity had 100% prevalence of infection while those that had moderate viscosity had lower prevalence of infection. For the fertile men, all had moderately viscous sperm since there was no presence of infection. This is to say that increase in infection leads to abnormal viscosity. This agrees with a work by Merino *et al* (2005); he observed that presence of infection in semen leads to high semen viscosity. Related studies elsewhere (Sanocka-Maciejewska *et al* 2005) also associated bacterial infection with human semen hyper viscosity.

Table 4 shows that sperm cells that had higher pH had higher percentage of active motility amongst the non fertile men. This is peculiar with results obtained from the fertile men. This also shows that higher pH encourages a higher motile activity by sperm cells. This is similar with a work by Ingerman *et al* (2002), which came to the conclusion that exposure of sperm to low pH buffer declines motility by 50%. This was also observed by Carr *et al* (1985); he observed effects of pH on sperm motility among different mammalian species. He observed that little or no motility was noted when sperm were incubated in vitro in their Cauda Epididymal fluid (CEF).

Table 1: Frequency of bacterial isolates

ISOLATES	Number (%)
Staphylococcus aureus	160 (39.2)
Klebsiella sp	56 (13.7)
Escherichia coli	40(9.8)
Proteus mirabilis	64(15.7)
Neisserria gonorrhoeae	36(8.8)

Table 2: Prevalence of Infection in relation to sperm count

Sperm Count	Non Fertile Men		Fertile Men	
	No. Examined	Prevalence of infection (%)	No. examined	Prevalence of Infection (%)
No Cell	16	100	0	0
< 20 Million	32	100	0	0
21-30 Million	168	100	0	0
40 Million	84	61.9	30	0

Table 3: Prevalence of infection in relation to Viscosity

Viscosity	Non Fertile Men		Fertile men	
	Number Examined	Prevalence of Infection (%)	Number Examined	Prevalence of Infection (%)
Watery	100	100	0	0
Slightly Viscous	100	100	0	0
Moderately Viscous	172	81.4	30	0
Highly Viscous	16	100	0	0

Table 4: Relationship of pH to sperm motility

pH	Non Fertile Men				Fertile Men			
	No. Examined	%) Active	% Sluggish	% Non Motile	No. examined	% Active	% Sluggish	% Non Motile
6.0	16	0	30	70	0	0	0	0
6.5	76	10	50	40	0	0	0	0
8	296	70	10	20	30	100	0	0

Discussion

Analysis of semen of fertile men and non fertile men has been evaluated. Five bacterial isolates were revealed indicating *Staphylococcus aureus* as the most predominant of other organisms which include *Klebsiella* sp., *Escherichia coli,Proteus mirabilis* and *Neisseria gonorrhoeae* for results obtained from subjects with fertility problem, while non was isolated from subjects without fertility problem. This result has shown that presence of microorganism is as a result of microbial infection and this is a major cause of damage to the spermatozoon cells. Other studies have shown that microbial infection affects sperm motility (Gomez *et al*., l979; Ekhaise *et al.,*2008). In the work of Ekhaise *et al*.(2008),50 semen samples of men with fertility problem were analyzed and *Staphylococcus aureus* (77.8%) was the most predominant species. Ikeagwu *et al. (*2008) also carried out microbial analysis on l74 semen samples where *Staphylococcus aureus* (66.7%) was the most prevalent.

It was also shown in this study that among the non fertile men, those that had sperm count below 40 million had 100% prevalence of infection while those that had sperm count above 40 million had lower prevalence of infection. Among the fertile men, no infection was recorded and as such all the men had sperm count above 40million.This is to say that sperm count increases as infection decreases. In other words microbial infection has a deteriorating effect on sperm concentration. This concords with a work carried out by Sanocka- Maciejewska *et al* (2005). In their work, it was revealed that there was a statistical significant deterioration in sperm concentration with increase in infection. This conclusion was arrived at when ejaculated samples of patients were analyzed for genital tract infection.

This study reveals that among the non fertile men, those that had extreme viscosity had 100% prevalence of infection while those that had moderate viscosity had lower prevalence of infection. For the fertile men, all had moderately viscous sperm. This is to say that highly viscous sperm may be as a result of the microbial infection. This agrees with a work by Merino *et al.* (2005); he observed that presence of infection in semen leads to high semen viscosity. Also studies by Sanocka-Maciejewska *et al* (2005) coraboroted that bacterial infection has a direct effect on human semen hyper viscosity.

This study has also shown that sperm cells that had higher pH had higher percentage of active motility both for the non fertile men and the fertile men. This was more peculiar with results obtained from the fertile men. This also shows that higher pH encourages a higher motile activity by sperm cells. This study has shown that an acidic environment may not be a conducive environment for the spermatozoa to thrive. This is similar with a work by Ingerman *et al* (2002) which came to the conclusion that exposure of sperm to low pH buffer reduced motility by 50%. This was also observed by Carr *et al* (1985); they observed effects of pH on sperm motility among different mammalian species and found that little or no motility was noted when sperms were incubated in vitro in their Cauda epididymal fluid (CEF).

Conclusion

This study has concord with other previous studies that poor parameters such as low pH, presence of micro-organisms in seminal fluid contributes to semen abnormalities. Presence of microorganisms in seminal fluid leads to poor sperm quality such as low sperm count, abnormal viscosity and motility. Sperm motility is essential for transport through the female reproductive tract and for fertilization. It is also an expression of the viability and structural integrity of the cell (Stephen *et al.* 1989). Studies also carried out by Auroux *et al*. (1991) indicated that it was probable that the presence of *E. coli* in semen decreases sperm

motility but that this depends on the sperm bacteria/ semen ratio/ ml. Gomez *et al.,* (1979) reported that microbial infections of the semen are major causes of male infertility. This study shows high percentage of bacterial isolates recovered from semen samples with poor semen data as well as the profound effect of micro-organisms on the semen motility. The negative influence of microorganisms towards sperm reproductive potential has been revealed in cases of infection with *E. coli* and *S. aureus* amongst other microorganisms (Sanocka-Maciejewska *et al*. 2005).

Infertility has posed so much problem in the society especially in Africa where so much attachment is given to children. Giving birth to children brings pride and fulfillment to couples. On the contrary this brings so much emotional and social problems in the society (Caldwell and Caldwell, 1978; CDC, 2000). Despite the increase in the prevalence of infertility, efforts have not been made in tackling this problem of assisting couples that are facing infertility. Instead government and international agencies are interested in controlling high growth rate (Romaniuk, 1969, Okonofuaa *et al.,* 1997). This study therefore suggests sex education, public health, hygiene awareness among men and control of sexually transmitted diseases. In any case of infection, one should contact a medical Doctor for evaluation and appropriate antimicrobial therapy.

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