

Full Length Research Paper

Common bacterial isolates associated with semen of men attending the fertility clinic of the university of Benin teaching hospital (U.B.T.H), Benin City, Nigeria

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Fifty 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 obtained 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 *S. 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 isolated bacterial. The motility of semen was recorded to be 20, 10 and 45% for *S. aureus*, *E. 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: Semen, bacterial isolates, men, infertility and Benin teaching hospital (UBTH), Benin City.

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 (Guyton, 1992). 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 accessory reproductive ducts and secretory glands (seminal vesicles, prostate gland and bulbourethral glands), which

are involved in the secretion of seminal fluid (Guyton, 1992). A few weeks before birth, the two developing testes pass out of the abdominal cavity into the scrotal sac (Bray et al., 1999). 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. 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 (Bray et al., 1999). 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

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Abbreviations: UBTH, University of Benin teaching hospital; HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; IVF, *in-vitro* fertilization; ARTs, assisted reproductive techniques.

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) (Vander et al., 1970). 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. 50% of men with counts of 20 to 40 million cells/ml and essentially all of those with count under 20 million cells/ml are sterile (Ganong, 2005). 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 (Stephen et al., 1989). Normal count is 20×10^6 spermatozoa/ml or more. Counts less than 20×10^6 cells/ml is known to be associated with male sterility. Normal spermatozoa measure 50 to 70 μm in length. Each consists of an oval-shaped head (with acrosomal cap), which measures $3\text{-}5 \times 2\text{-}3 \mu\text{m}$, 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 (WHO, 1999). Infertility constitutes a grave emotional and social problem in societies where great importance is attached to having children (Caldwell and Caldwell, 1978; CDC, 2000). 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 to 30% of couples in Africa experience primary and secondary infertility (Okonofua, 1999). The most affected areas lie within the central African region referred to as the "infertility belt" of Africa (Okonofua, 1999; Romaniuk, 1969). Although Nigeria does not fall within the infertility belt, there are indications of high and rising prevalence levels (CDC, 2000). 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 (Romaniuk, 1969; Widge, 2001, Okonofua et al., 1997). 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 (Gomez et al., 1979). 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 (Onemu and Ibeh, 2001). 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 to 7 days of sexual abstinence from intercourse, using the masturbation method. Upon collection, samples were transferred to the laboratory in a temperature that is as close as possible to body 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 $\text{ml} \pm 0.1$. 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 sec, 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 systemically 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.

Table 1. Frequency of bacterial isolates.

Group	Bacterial Isolate	Frequency	Percentage
A	<i>Staphylococcus aureus</i>	7	77.8
B	<i>Escherichia coli</i>	1	11.1
C	<i>Citrobacter</i> spp.	1	11.1

Count

Improved Neubauer Counting Chamber was used for the count. One twenty ($1/20$) dilution of semen was done with formol saline as diluents. Count = $N \times 10^6$ /ml.

Calculation

Depth = 0.1 mm

Area = $1/5 \times 1/5 = 1/25 \times 5 = 1/5 \text{ mm}^2$

Volume = $0.1 \text{ mm} \times 1/5 \text{ mm}^2 = 1/50 \text{ mm}^3$

If N cell is in $1/50$, $N = n \times 50 \times 20 = 1000$

Converting to ml,

$1000 \times 1000 = 10^6$ N. Dilution factor is 20.

Cultural method

Culture of seminal fluid samples (50) was done in aseptic condition, within 1 h of collection the seminal fluid was cultured using Blood Agar, Chocolate Agar and MacConkey Agar at 37°C for 24 h. 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 (Buchanan and Gibbons, 1978), Gerhardt et al., 1994). 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 10 µg), Tetracycline (TE 10 µg), Cloxacillin (OB 5 µg), Augmentin (Aug 30 µg), Amoxicillin (Amx 25 µg), Chloramphenicol (C 30 µg), Cotrimoxazole (SXT 25 µg), Erythromycin (E 5 µg).

RESULTS AND DISCUSSION

Fifty samples of semen collected from men attending the fertility clinic of the UBTH, Benin City, were examined routinely, for normal semen characteristics. Three genera of bacterial isolates were obtained and these include *Staphylococcus*, *Escherichia* and *Citrobacter*, with the highest counts recorded for the genera *Staphylococcus* 7(77.8%), *Escherichia* 1(11.1%) and *Citrobacter* 1 (11.1%) Tables 1 and 2 shows the distribution of the bacterial isolates relative to sperm concentration. Table 3 shows the recovery frequency of the bacterial isolates relative to semen characteristics. A high percentage of the isolates were recovered from semen samples with poor semen data. Table 4 shows the effect of microorganisms on the semen motility. The incidence of

infertility in Africa has assumed an alarming proportion as many couples experience this reproductive health problem (Ojobo, 2007). This has posed significant inhibition to many reproductive health interventions such as maternal health care, sexually transmitted diseases and Human Immunodeficiency Virus Acquired immune deficiency syndrome (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 (CDC, 2000).

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, (2000) it has been observed that 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 (Schmidt et al., 2005, Caldwell and Caldwell, 1978). 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 (Mogra et al., 1981). 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 ejaculation (Burkharin et al., 2000). Additionally, the identification of pathogenic microorganisms in a sterile semen culture may be of significance (Glover et al., 1990).

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 (Cottel et al., 2000). If the presence of micro-organisms 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 (Swenson et al., 1980).

In the study, it was reported that, the frequency of the bacterial isolates, particularly the genera *Staphylococcus*, *Escherichia* and *Citrobacter* were the most predominantly common bacterial isolates associated with the semen of men complaining of infertility. This report was supported by earlier reports by Sanocka-Macleiewska et al. (2005).

Among the bacteria isolates, the highest count was

Table 2. Distribution of isolates relative to sperm concentration.

Isolates	No	Sperm concentration ($\times 10^6$ /ml)		
		0.0	< 19.9	>20
<i>Staphylococcus aureus</i>	7	2	5	0
<i>Escherichia coli</i>	1	0	1	0
<i>Citrobacter</i> spp.	1	0	0	1
Total	9	2	6	1

Table 3. Recovery frequency of organisms.

Classification	No of samples	No of organisms	Percentage
Azoospermia	5	2	40
Oligospermia	29	6	20.68
Normozoospermia	16	1	6.25

Table 4. Sperm motility in relation to bacterial isolates.

Identity of Isolate	No of cases	Total (%)	Progressive (%)
<i>Staphylococcus aureus</i>	7	30.71	20
<i>Escherichia coli</i>	1	20	10
<i>Citrobacter</i> spp.	1	55	45
Samples without significant growth	41	39.5	29.14

recorded for genera *Staphylococcus* 7(77.8%). The presence of the microorganisms is an indication of microbial infection. Gomez et al. (1979) reported that, microbial infections of the semen are major causes of male infertility. In the study, 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. (1989) 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 *E. coli* and *S.aureus* amongst other microorganisms (Sanocka et al., 2005). Studies 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 bacterial/semen ratio/ml.

A study carried out by Huwe et al. (1998) revealed that a variety of pathogenic microorganisms, *Candida albicans* inclusive, exert significant inhibitory effect on spermatozoa motility. Bornman, (1990) established that *Ureaplasma urealyticum* affect fertility and *Mycoplasma hominis* is known to cause tail abnormalities of spermatozoa. They have been found in significant amount in the semen of some infertile men. In a study carried out in Nigeria, Alausa and Osoba (1978) 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 (for example, ozone) that disrupt sperm production or the function of reproductive hormones or sperm may increase the risk of male infertility (Sokol et al., 2006). Previous studies by Alausa and Osoba (1978) 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 anti-microbial therapy (Yeboah et al., 1992). 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 (Ogunbanjo et al., 1989). 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 (Damirayakhain et al., 2006). Occupational exposures to heavy metals for example, lead, zinc and arsenic have been reported to impair spermatogenesis (Coste et al., 1991). In addition, the effects of specific herbicides and pesticides have also been found to be toxic to spermatogenesis (Eaton et al., 1986).

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 (Hollos, 2003; Caldwell and Caldwell, 1978; CDC, 2000). 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 (Okonofua, 2003). This is because most attempts aimed at the surgical treatment of infertility have achieved limited success (Ojobo, 2007). 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 (2007) has proffered 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

- Guyton AC (1992). Human Physiology and Mechanisms of Diseases. 11th edition. Elsevier, London.
- Bray JJ, Cragg PA, Mac Knight AD, Mills RG (1999). Human physiology. (4th edition), University Press Cambridge Great Britain, p. 610.
- Vander AJ, Sherman JH, Luciano DS (1970). Human Physiology. The Mechanisms of Body Function. 4th edition. McGraw Hill Company, p. 715.
- Ganong WF (2005). Review of Medical Physiology. 22nd Edition. Elsevier, Philadelphia.
- Stephen PB, Rusell OD, David FK (1989). Current problems in obstetrics, gynaecology and fertility. Yearbook Medical Publishers, Incorporated, p. 199.
- World Health Organization (1999). Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4th edition. Cambridge University Press, Cambridge, p. 99.
- Caldwell JC, Caldwell P (1978), "A Theory of Fertility. From High Plateau to Destabilization" Population and Development Review. No. 4.
- Centers for Disease Control and Prevention (CDC) (2000). Family planning methods and practice. Africa. United States Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Control and Health Promotion, Division of Reproductive Health, pp. 153 – 179.
- Okonofua F (1999). Infertility and women's reproductive health in Africa: Afr. J. Reprod. Health, 13(1): 7-8.
- Romaniuk A (1969) "Infertility in Tropical Africa". In: Okonjo, C. and Caldwell, J.C. The Population of Tropical Africa. Longman Press, London, p. 233.
- Widge A (2001). "Socio-cultural attitudes towards infertility and assisted reproduction in India" In: Current Practices and Controversies in Assisted Reproduction Report. World Health Organization (WHO), Geneva, Switzerland, p. 74.
- Okonofua FE, Harris D, Odebiyi A, Kane T, Snow RC (1997). "The Social Meaning of Infertility in South West Nigeria" Health Transition Review. 7.
- Gomez CI, Stenback WA, James AN, Criswell BS, Williams RP (1979). Attachment of *Neisseria gonorrhoea* to human sperm. Microscopical study of the effect of trypsin and iron. Brit. J. Ven. Dis., 55: 245 – 255.
- Onemu SO, Ibeh IN (2001). Studies on the significance of positive bacterial semen cultures in male infertility in Nigeria. Internat. J. Fert. Women Med., 46(4): 210 – 214.
- Buchanan RE, Gibbons EN (1978). Bergey's Manual of Determinative Bacteriology. 8th edn. Williams and Wilken. Co., Baltimore, p. 1300.
- Gerhardt P, Murray RGE, Wood WA, Krieg NR (1994). Methods for General and Molecular Bacteriology. ASM Press, Washington DC, p. 791.
- Ojobo S (2007). Management of infertility amongst Nigerian couple: The way forward. Women's Health and Action Research Center. Community Education Lecture Series. Benin City. 26pp.
- Schmidt J, Hodsten BE, Christentein U, Bovin U (2005). The social epidemiology of coping with infertility" Human Reprod., 20: 1044 – 1052.
- Mogra N, Dhruva A, Kothan LK (1981). Non-specific seminal tract infection and male infertility. J. Postgrad. Med., 27(2): 99 – 104.
- Bukharin OV, Kuzmin MD, Ivanovi UB (2000). The role of the microbial factor in the pathogenesis of male infertility. Zeifungwiss. Mikrobiol. Epidemiol. Immunobiol., 2: 106 – 110.
- Glover TD, Barrett CL, Tyler JP (1990). Human Male Fertility and Semen Analysis. Academic Press, London, p. 247.
- Cottell E, Harrison RF, Mc Caffrey M, Walsh T, Mallon E, Barry – Kinsella C (2000). Microorganisms are commonly found in insignificant quantities in the semen of asymptomatic men. Fert. Steril., 74(3): 465 – 470.
- Swenson CE, Toth A, Toth C, Wolfgruber L, O'Leary WM (1980). Asymptomatic bacteriospermia in infertile men. Androl. 12(1): 7 – 11.
- Sanocka – Macleiewska D, Ciupinska M, Kurpis ZM (2005). Bacterial infection and semen quality. J. Reprod. Immunol., 67(1-2): 51 – 56.
- Auroux MR, Jacques L, Mathieu D, Auer J (1991). Is the sperm bacterial ratio a determining factor in impairment of sperm motility: an *In-vitro* study in man with *Esherichia coli*. Internat. J. Androl., 14(4): 264 – 270.
- Huwe P, Diemer T, Ludwig M, Liu J, Schiefer HG, Weidner W (1998). Influence of different uropathogenic microorganisms on human sperm motility parameters in an *in-vivo* experiment. Andrology, 30(suppl 1): 55-59.
- Bornman MS (1990). Microbial flora in semen of infertile African men at Gasrankuwa hospital. J. Androl., 22(2): 118 – m121.
- Alausa KO, Osoba AO (1978). The role of sexually transmitted diseases in male infertility in Tropical Africa. J. R. Soc. Med., 8(3): 225 – 229.
- Sokol RZ, Kraft P, Fowler IM, Memet R, Kim E, Berhane TK (2006). Exposure to environmental ozone alters semen quality. Environ. Health Perspect, 114(3): 360 – 365.
- Yeboah EO, Wadhvani JM, Wilson JB (1992). Etiological factors of male infertility in Africa. Internat. J. Fert., 37(5): 300 – 307.
- Ogunbanjo BO, Osoba AO, Ochei J (1989). Infective factors of male infertility among Nigerians. Afr. J. Med. Sci., 18(1) 35 – 38.
- Damirayakhain M, Jeyendran RS, Land SA (2006). Significance of semen cultures for men with questionable semen quality. Arch. Androl., 52(4): 239 – 242.
- Coste J, Mandereau L, Pessione (1991). Lead-exposed workman and fertility: A cohort study on 354 subjects: Euro. J. Epidemiol., 7: 154 – 158.
- Eaton M, Schenker M, Whorton D (1986). Seven-year follow-up workers exposed to 1,2-dibromo-3-chloropropane. J. Occupat. Med., 28: 1145 – 1150.
- Hollos M (2003). Profiles of Infertility in Southern Nigeria: Woman's voice from Amakiri. Afr. J. Reprod. Health, 7(2): 10 -13.
- Okonofua FE (2003). "New Reproductive Technologies and Infertility Treatment in Africa". Afr. J. Reprod. Health, 7(1): 7-8.