EVALUATION OF THE RELATIONSIP BETWEEN LEUCOCYTOSPERMIA AND BACTERIOSPERMIA IN SEXUALLY ACTIVE MALES IN BENIN CITY, NIGERIA.

[1]Onemu, S.O, [2] Ophori, E.A*, [2] Ogbimi, A.O.

[1]Department of Medical Microbiology, University of Benin Teaching Hospital, P.M.B. 1111, Benin City, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria.
Corresponding Author: Tel: + 2348023393102 , Email : eaophori@yahoo.com

ABSTRACT

The presence of leucocytes and bacteria in spermatozoa is known as leucocytospermia and bacteriospermia respectively. In sexually active males, both conditions tend to reduce semen quality. This study was carried out to determine the presence and effects of leucocytes and bacteria in semen of sexually active males in Benin City,Nigeria. A total of 187 sexually-active men attending the University of Benin Teaching Hospital, Benin City, Nigeria volunteered to participate in the study. They were aged 19-33 years (mean = 23.7 ± 3.2) .Seminal fluid samples were submitted by participants after 3 days abstinence from alcohol, drugs and sex. The samples were cultured on bacteriological media and identified by standard methods. Spermatozoa concentration, leucocytes density, motility and other parameters were performed microscopically using the World Health Organization guidelines. Antimicrobial sensitivity was done on isolates by the disc diffusion method. Out of 187 samples, 70 (37.4%) were positive for bacteriospermia. *Staphylococcus aureus* had the highest prevalence of 57.1%, this was followed by *Escherichia coli* (15.7%), *Mycoplasma* sp.(14.3%), *Klebsiella* spp. (7.1%) and *Candida albicans* (5.7%). Leucocyte counts ranged from 2.9-6.0 x 10^6/ ml. There was an increase in bacteriospermia with increase in leucocyte counts. When leucocyte counts were $\geq$ 3 x 10^6/ml, the percentage of pathogenic microorganisms isolated and the progressive motility increased significantly ($P < 0.001$) for all the isolates except for *Mycoplamsa* spp. Antimicrobial sensitivity of all isolates (except *Candida albicans*) was higher to ciprofloxacin and pefloxacin. Although leucocytes is a poor marker of bacteriospermia, leucocytes density of $\geq 3x10^6$/ml was a threshold marker for positive bacterial semen culture in this study. The presence of leucocytospermia and bacteriospermia were strongly associated with poor semen quality.

Key words: *Leucocytes, Leucocytospermia, bacteriospermia, semen quality, sexually active males.*

Introduction

Leucocytospermia may often be attributed to male genital tract infection (Wang *et al.*,1994). Elevated levels of leucocytes in semen have been associated with poor semen quality (Wolff *et al*., 1990; Yanushposky *et al*.,1990), or compromised sperm structural integrity (Aziz *et al*.,2004).

The prevalence of leucocytospermia could vary widely in different populations depending on factors such as sexual practices, the prevalence of sexually transmitted pathogens (Wolff, 1995) and from the percentage of the DNA of sexually transmitted pathogens detected in asymptomatic male infertility cases with poor semen quality (Benzold *et al.*,2007). Leucocytospermia has been reported as the most common cause of male infertility but the distribution, origin and role of leukocytes in semen is still considered controversial or having poor sensitivity and specificity(Onemu and Ibeh, 2001). Leucocytospermia is regarded as a poor marker of bacteriospermia because of the lack of correlation between positive semen cultures and sperm characteristics (Rodin *et al.*,2003). There is however, increasing evidence to suggest that there is a relationship between leukocytes and poor semen parameters (Yanushposky *et al*.,1990). The role of leucocytes in semen is thought to be associated with oxidative stress because leucocytes are major producers of reactive oxygen species (ROS) which damage spermatozoal function (Sharma *et al.*,2001; Li and Liu,2006) or related to the functional competence of spermatozoa (Aitkin *et al.*,1994). Leucocytes concentration >$2x10^6$/ml has been suggested as the most likely to be unfavour-

able to the quality of semen profile and has been reported to contribute to the increasing rate of infertility amongst sexually active men (Kaleli *et al*.,2000).This study was therefore designed to evaluate the relationship between leucocytes and micro-organisms in semen of sexually active men in Benin City.

PATIENTS AND METHODS

Study Population: Seminal fluid samples from volunteer sexually-active young men aged 19–33 years (mean 23.7± 3.2) who gave oral consent after the aims and objective of the study was explained to them by the clinical physician who assisted in the study. Each participant (volunteer) was advised to abstain from passing semen for 72 h, wash hands and genitals with mild-toilet soap and dry skin with a sterile disposable towel provided.

Sample collection and Examination: Semen was collected by physical masturbation by each participant for delivery to laboratory within 1h of collection. Each seminal fluid was serially diluted ten-fold in physiological saline (NaCl 0.15M). Inoculation of 0.1ml of diluted semen was done on blood agar (Oxoid CM 55) heated blood agar (Oxoid CM 55), MacConkey agar (Oxoid CM7), Gonococcal agar (Lab 39), Sabouraud dextrose agar (Oxoid CM 41) and heart agar enriched with 30% serum and incorporated with 60mg ceftriaxone. All inoculated culture media were incubated at 37^0C for 24 -48 h. Cultures for *Mycoplasma* spp. were incubated for 96 h.

Cultures with colony forming units (cfu) $\geq 10^3$/ml of a single microbial type were picked for cultural, microscopical and biochemical characterization and identification (Cowan, 1974). Sensitivity tests were carried out on the bacterial isolates by the disc diffusion method [Baker and Breach,1980). The seminal fluid samples were also examined for spermatozoa density and leukocyte count using standard methods (WHO,1999).

Data generated were analyzed using one-way analysis of variance (ANOVA) to compare leukocyte count and spermatozoa density of semen with pathogenic micro-organisms to those without pathogens (Ogbeibu, 2005).

RESULTS

The examination of 187 semen samples from volunteer sexually active young men in Benin City, Nigeria resulted in the isolation of 70 (37.4 %) potentially pathogenic micro–organisms.

Table 1 shows the comparison of the frequency of isolated micro-organisms, leucocytes counts and spermatozoa density. *Staphylococcus aureus* was the most prevalent organism, followed by *Escherichia coli* 11 (15.7%), *Klebsiella* spp. 5(7.1%), *Candida albicans* 4(5.7%) and *Mycoplasma* spp. 10 (14.3%). The leucocytes count from seminal fluid samples with potentially pathogenic microorganism were significantly higher ($p< 0.001$) than those without pathogens and mycoplasmal isolates. The progressive motility percent of spermatozoa from semen with pathogens was significantly lower ($p<0.001$) with exception of samples that yielded *Mycoplasma* species. The leucocytes count and spermatozoa concentration from seminal fluid samples with *Mycoplasma* isolates were not significantly different ($P>0.05$) from semen without pathogenic isolates.

The antimicrobial sensitivity test on bacterial isolates is shown in Table 2 . The isolates were generally more susceptible to ciprofloxacin and pefloxacin. However, superior activity was shown against *Staphylococcus aureus* with amoxycillin-clavulanate while no activity was demonstrated against ampicillin and tetracycline respectively.

Fig. 1 shows the comparison of leukocytes and recovery of micro-organisms. The percentage of micro-organisms recovered was 1.4% from leucocytes count $\leq 2.9x10^6$/ml while the percent of pathogenic isolates recovered increased significantly ($P<0.001$) to 7.1% with a leucocytes count of 3.0- 3.9 $x10^6$/ml. The highest percent (77.1%) of isolates were from semen samples with leucocytes count 4.0–5.9 x 10^6/ml.

Table 1: Comparison of bacterial isolates frequency, leucocytes count and spermatozoa concentration

Microoganisms	Percentage of isolate	Mean leucocyte count (x10^6/ml)	Mean spermatozoa concentration (x10^6/ml)	Mean progressive motility percent
Staphylococcus aureus n = 40	57.1	4.9	17.0	35.0
Escherichia coli n = 11	15.7	5.4	33.3	38.3
Klebsiella spp. n = 5	7.1	4.7	12.7	25.0
Candida albicans n = 4	5.7	3.3	7.5	18.5
Mycoplasma spp. n = 10	14.3	0.6	76.8	65.0
Other semen samples n = 115	0.0	0.5	79.2	66.5

Table 2: Antimicrobial sensitivity of bacterial isolates

	Antimicrobial agent							
Bacterial isolate	**Genta mycin**	**Peflox acin**	**Oflox acin**	**Azithro mycin**	**Amoxycillin clavulanate**	**Cloxa cillin**	**Amoxa cillin**	**Tetracy cline**
Staphylococcus aureus n = 40	70.0	90.0	90.0	77.5	75.6	97.5	0.0	0.0
Escherichia coli n = 10	45.5	91.0	91.0	60.0	63.3	40	0.0	0.0
Klebsiella spp. n = 5	40.0	60.0	60.0	40.0	60.0	0.0	0.0	0.0

Each value represents the percentage of the number of isolates for a particular organism

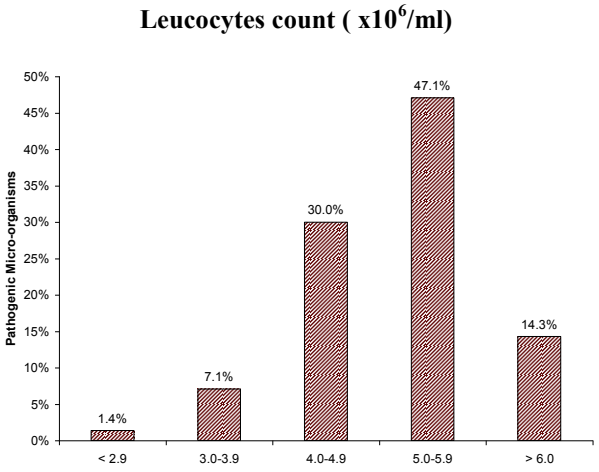

Fig 1: *Distribution of pathogenic micro-organisms relative to leucocyte count.*

DISCUSSION

The presence of pathogenic micro-organisms (bacteriospermia) and elevated leukocyte count (leucocytospermia) may represent an important indicator in the deterioration of semen parameters. The examination of semen from 187 volunteer sexually-active young men, aged 19–33 ($\bar{x}$ = 23.7±3.2) years in Benin City, Nigeria resulted in the isolation of 70 (37.4%) microorganisms. *Staphylococcus aureus* 40 (57.10%) was the most frequently recovered micro-organisms. This observation is in agreement with earlier reports on semen microbial quality (Giamarellou *et al*.,1984; Onemu and Ibeh,2001; Emokpae *et al*.,2005). Other isolated micro-organisms were *Escherichia coli* 11(15.7%), *Mycoplasma* spp. 10 (14.3%), *Klebsiella* spp. 5 (7.1%).These micro-organisms have been reported to be associated with urogenital tract infections in both males and females (Brooks *et al*.,2004). *Candida albicans* 4 (5.7%) was the least frequently isolated micro-organisms, paradoxically, it was recovered from seminal fluid samples with the most severe signs of deterioration. This suggests that *Candida albicans* may represents a serious hazard to male fertility when semen is infected (Witkin and Toot,1983; Brooks *et al,*2004). The role of *Mycoplasma* spp. in semen quality was not determined as had been previously reported (Kjaergaad *et al.*,1997;Keck *et al.*,1998].

The mean value for leucocytes count from semen with mycoplasmal isolates was significantly lower ($P < 0.001$) when compared with semen samples with pathogenic isolates. Similarly, the mean spermatozoa density for these samples was also significantly higher ($P < 0.001$). However, the mean leucocyte counts and spermatozoa densities were not significantly different ($p>0.05$) for semen samples without pathogenic micro-organisms. It may thus be suggested that *Mycoplasma* spp. in the semen samples may be opportunistic colonizers. The role of this micro-organism in semen may need further evaluation.

The bacterial isolates were generally more susceptible in the *in-vitro* test to ciprofloxacin and pefloxacin. The high antimicropbial activity of these agents in similar situations has been reported (Andriole, 1991; Childs,1991). The superior activity shown by amoxycillin-clavulanate against *Staphylococcus aureus* suggests that this antibiotic remains the most effective in managing *Staphylococcus aureus* infections as previously reported (Piroth *et al*.,2009).Lack of activity with ampicillin and tetracycline may have been largely influenced by the common misapplication and misadministration of these agents in the study community.

The number of pathogenic micro-organisms isolated with leucocytes count of $\geq$3.0–5.9 x 10^6/ml increased significantly ($P < 0.001$). The highest frequency of pathogenic micro-organisms recovery was from leukocyte

count range of 3.0–3.9 x 10^6/ml where as lower number of such micro-organisms was recovered from leukocytes counts $\geq$ 6 x 10^6/ml. This reinforces the observation that leukocyte count is not an adequate or sensitive marker of bacteriospermia.

Conclusion:

Leucocyte of $\geq 3x10^6$/ml represents a threshold for positive bacterial semen culture or bacteriospermia in this population. The concurrent presence of leucocytospermia and bacteriospermia were strongly associated with decreased semen quality.

ACKNOWLEDGEMENT

The authors are sincerely grateful to all the male participants in this study.

REFERENCES

Aitkin, R.J., West K and Buckingham, D (1994). Leukocytic infiltration into human ejaculate and its association with semen quality, oxidative stress and sperm function. *Journal of Andrology* **15(4):** 343–352.

Andriole, V.T.(1991) Use of quinolones in the treatment of prostatitis and lower urinary tract infections. *European Journal of Clinical Microbiology and Infectious Diseases* **10**(4): 342–350.

Aziz, N., Agarwal, A., Lewis-Jones, I., Sharma, R.K. and Thomas, N. (Jr.) (2004) . Novel associations between specific morphological defects and leukocytospermia. *Fertility and Sterility* **82 (3):** 621–627.

Baker, F.J and Breach, M.R (1980). Antibiotic Susceptibility Testing *In*: *Medical Microbiological Techniques* (1st ed.), Butterworths, London. 643pp.

Benzold, G., Politch, J.A., Kiviat, N.B., Kuypers, J.M., Woff, H. and Anderson, J.D.(2007) . The prevalence of sexually transmitted pathogens in semen from asymptomatic male infertility patients with and without leukocytospermia. *Fertility and Sterility* **87(5):** 1087–1097 .

Brooks, G.F., Butel, J.S. and Morse, S.A. (2004). *Jawetz, Melnick* and *Adelberg's Medical Microbiology* (12th ed.) McGraw Hill, Boston.

Childs, S.J. (1991) Ciprofloxacin on treatment of chronic bacteria prostatitis *Urol*ogy **35 (suppl):** 15 – 18.

Cowan, S.T. (1974) .*Cowan and Steels Manual for the Identification* of *Medical Bacterial* (2nd ed), Cambridge University Press, London.

Emokpae, M.A, Uadia, P.O and Saidiq, M.M. (2005). Male Infertility: Semen quality and infection in Kano, Nigeria *Journal of Medicine and Biomedical Research* **4(2):** 34-38.

Giamarellou, H., Typanidis, K., Bitos, N.A., Leonidas, E., Daikos, G.K. (1984). Infertility and chronic prostatitis. *Andrologia.* **16 (5):** 417-422.

Kaleli, S., Ocer, F., Irez, T., Budak, E., and Aksu, M.F. (2000) . Does Leukocytospermia associate with poor semen parameters and sperm functions in male fertility? The role of different seminal leukocyte concentrations. *European Journal of Obstetrics and Gynecology and Reproductive Biology* **89(2):** 185–191.

Keck, C., Gerher-Schafer, C., Clad, A., Wilheim, C. and Breckwoldt, M. (1998). Seminal tract infections: Impact on male fertility and treatment options. *Human Reproduction Update* **4(6):** 891 -903.

Kjaergaard, N., Kristensen, B., Hansen, E.S., Farholt, S., Schonhegder, H.C., Uldberg, N. and Madsen, H.(1997) Microbiology of semen specimens from males attending a fertility clinic. *Acta Pathologica Microbiologica Et Immunologica Scandinavica* **105(7):** 566-70.

Li, J. and Liu, R.Z. (2006) . Progress in Leukocytospermia research. *Zhonghua Nan Ke Xue.* **12(8):** 730–736.

Ogbeibu, A.E.(2005).*Biostatistics:A Positive Approach to Research and Data Handling,* Mindex Publishing Co. Ltd., Benin City.

Onemu, S. and Ibeh, I.N (2001) Studies on the significance of positive bacteria semen cultures in male infertility in Nigeria. *International Journal of Fertility*

and Women's Medicine **46 (4):** 210–14.

Piroth, L., Prchinot, A., Minello, A., Jaulhac, B., Patry, I., Hadou, T., Hansmann, Y., Roaud, C., Chavamet, P. and Neuwirth, C.(2009) Bacterial epidemiology and antimicrobial resistance in ascitic fluid in 2-year retrospective study. *Scandinavian Journal of Infectious Diseases*. **41(11-12):** 847-851

Rodin, D.M., larone, D. and Goldstein, M. (2003). Relationship between semen cultures leukocytosperma and semen analysis in men undergoing fertility evaluation. *Fertility and Sterility* **4 (Supp 3):** 1555 –1558.

Sharma, R.K., Pasqualotto, A.E., Nelson, D.R., Thomas, A.J. (Jr) and Agarwal, A.(2001) Relationship between seminal white blood cell counts and oxidative stress in men treated at an infertility clinic. *Journal of Andrology* **22(4):** 475 –583.

Wang, A.W., Politch, J. and Anderson, D. (1994). Leukocytospermia in male infertility patients in China. *Andrologia.* **26 (3):** 167 – 172.

Witkin S.S. and Toot A (1995). Relationship between genital tract infection, sperm antibodies in seminal fluid and infertility. *Fertility and Sterility* **40(6):** 805–806.

Wolff, H. (1995). The biologic significance of white blood cells in semen. *Fertility and Sterility* **63 (6):** 1143 –1157.

Wolff, H., Politch, J .A, Martinez, A., Haimvici, F., Hill, J.A. and Anderson, J.D. (1990) . Leukocytospermia with poor semen quality. *Fertility and Sterility* **53**(3): 528 – 536.

World Health Organization (1999). *World Health Organization Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction (*4th ed). Cambridge University Press, Cambridge.

Yanushposky, E.H, Politch, J.A, Hill, J.A and Anderson, J.D. (1990). Is leukocytospermia clinically relevant? *Fertility and Sterility* **66 (5):** 822–825.