Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.3, No.20, 2013

Relationship between Leukocytospermia and Sperm Count in Men Visiting Fertility Clinic of the Federal Medical Center Yenagoa Bayelsa State Nigeria.

AGORO ENI-YIMINI SOLOMON (Corresponding author)
DEPARTMENT OF CHEMICAL PATHOLOGY, MEDICAL LABORATORY SERVICES, FEDERAL MEDICAL CENTER, YENAGOA, BAYELSA STATE, NIGERIA.
+2348037434995 siragoro@yahoo.com

OGREGADE ILEIMOKUMO E
BAYELSA STATE AGENCY FOR THE CONTROL OF HIV/AIDS, YENAGOA, BAYELSA STATE
+2348036745226, ogregadeie@yahoo.com

MIEEBI M WANKASI
DEPARTMENT OF MEDICAL LABORATORY SCIENCE, COLLEGE OF HEALTH SCIENCES, NIGER DELTA UNIVERSITY, BAYELSA STATE, NIGERIA.
+2348064041614, mieebiwankasi@yahoo.com

ABSTRACT
Sperm count, or *sperm concentration* measures the concentration of sperm in a man's ejaculate, distinguished from *total sperm count*, which is the sperm count multiplied with volume. Over 15 million sperm per milliliter is considered normal, according to the WHO in 2010. Older definitions state 20 million. Sperm count is the focal factor of conception and is threatened by variety of intrinsic and extrinsic factors. Leukocytospermia is been argued as one factor that decrease sperm count and quality. An abnormally high concentration of WBC's in the semen is a condition known as leukocytospermia, also referred to as leukospermia, pyospermia or pyosaemia. This research was designed to establish if a relationship do exists between seminal sperm count and leukocytospermia. One hundred (100) apparently healthy men visiting the Microbiology Department of the Federal Medical Centre, Yenagoa for semen analysis from August 2012- March 2013 constituted the study population. The study population was divided into two groups; non-leukocytospermic subjects and leukocytospermic.The sperm count was assessed by improved bright-light Neubauer haemocytometer. The research carried out correlating the relationship between sperm count and leukocytospermia was statistically significant ($P<0.05$). Leukocytospermic samples tend to have decreases sperm count as compared to non leukocytospermic samples. Also cases of aspermia were more rampant in leukocytospermic subjects than non-leukocytospermic subjects. Leukocytospermia is a signal of inflammation and infection of the male reproduction apparatus and hence Medical Practitioners should matter of urgency employ the appropriate treatment regime to eliminate the causative agent.
Key words: Leukocytospermia, non-leukocytospermic , sperm count, seminal fluid, MAGI : Male accessory sex gland infection,spermatozoa.

Introduction

Semen is an organic fluid, also known as *seminal fluid*, that may contain spermatozoa. It is secreted by the gonads (sexual glands) and other sexual organs of male or hermaphroditic animals and can fertilize female ova. In humans, seminal fluid contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose are elements of seminal fluid which promote the survival of spermatozoa and provide a medium through which they can move or "swim".Semen is produced and originates from the seminal vesicle, which is located in the pelvis. The process that results in the discharge of semen is called *ejaculation*.
Semen analysis is the first line laboratory investigation for the assessment of male fertility competence. The examination and evaluation of certain established semen parameters can provide key information about the quality of a patient's semen and the functional competence of the spermatozoa[1]. The central necessity for seminal analysis is anchored on the sperm count. Sperm count refers to the amount of sperm per milliliter of semen collected. The amount of semen per ejaculation can vary quite a bit. Anywhere from less than a milliliter to 6 or 7 milliliters is considered normal. Sperm count, or *sperm concentration* measures the concentration of sperm in a man's ejaculate, distinguished from *total sperm count*, which is the sperm count multiplied with volume.[2.] Over 15 million sperm per milliliter is considered normal, according to the WHO in 2010.[3] Older definitions state 20 million.[4,5]

A lower sperm count is considered oligozoospermia. The average sperm count today is between 20 and 40 million per milliliter in the Western world, having decreased by 1-2% per year from a substantially higher number decades ago.[6]. However, an abnormally high concentration of WBC's in the semen is
a condition known as leukocytospermia, also referred to as leukospermia, pyospermia or pyosaemia.[7] The WHO laboratory manual's guidelines define leukocytospermia as the presence of $=1x10^6$ WBC's per milliliter of semen.[7]
An increased concentration of leukocytes is a molecular defense mechanism against the presence of foreign organisms and the detection of pathologicalconcentrations of leukocytes, with the exclusion of a bladder infection or urethritis, has been suggested as a basic diagnostic tool in recognizing MAGI [8,9].
The prevalence of leukocytospermia in sample population groups has yielded varying results. In a study by Kaleli et al.[10],
up to 72% of the patients examined were found to be suffering from the condition. At present, due to the fact that the condition is asymptomatic and various sites in the reproductive system can be affected[11] the exact site of the origination of
excess leukocytes is unknown [12, 13].
Due to the discrepancy as to the exact etiology of these cells, their release may be initially prompted by an inflammatory response of the genital tract to a bacterial invasion and then continually produced in their absence by immunological activity[14].
Generally leukocytospermia (WBC in the semen) affects 5-10% of the patient population, but can rise to 20% in certain patients group. WBC cells are deleterious because of their ability to stimulate the release of reactive oxygen species (ROS), thereby inhibiting sperm motility and sperm function. Reactive oxygen species (ROS) are produced by polymorphonuclear cells .The three main ROS are superoxide anion, hydrogen peroxide, and the hydroxyl radical. On the other hand, seminal plasma contains a number of antioxidants that protect sperm from oxidative damage from exposure to ROS. Men who have higher concentrations of such antioxidants may be able to tolerate greater concentrations of seminal leukocytes. Despite an apparently abnormal threshold level for leukocytes within the semen, a wide range of conflicting evidence exists as to the significance of seminal leukocytes and infertility. The impact of this condition and its treatment on semen quality are extremely controversial. The association between seminal leukocytes and semen quality is still a matter of debate in the literature. Leukocytospermia, defined by the World Health Organization (WHO) as more than 1 million leukocytes per millilitre, has an incidence of 10-20% in the general population and is especially common in infertile men. However, lower concentrations of seminal leukocytes (0-1 × 106/mL) are still more widespread, and are seen even in the absence of infection [7,5,15,16,2,3].

As leukocytospermia is so prevalent in infertile men, it can be questioned as to whether the presence of seminal leukocytes correlates with sperm count. Early studies found leukocytes to have a positive effect on semen quality [17,18].Tomlinson et al. reported that leukocytes phagocytosed abnormal spermatozoa [17]whereas Kiessling et al. found an improvement in sperm motility in semen samples with a leukocyte concentration of >2 × 106/mL[18] . However, the results of more recent studies suggest that leukocytes negatively impact on semen quality as a result of the presence of reactive oxygen species (ROS), which are primarily produced by leukocytes. It is believed that ROS are harmful to spermatozoa [19,20,21,22,12]. Aziz et al. reported a positive correlation between leukocytospermia and sperm tail defects, acrosomal damage and high sperm deformity index scores [12]]. However, in another study, Ziyyat et al. [23] reported an increase in sperm motility in semen samples with moderate leukocytospermia (defined as seminal leukocytes <1 × 106/mL), but observed a paradoxical decrease in sperm motility in semen samples exceeding a threshold of 1 × 106 leukocytes/mL. Similar results were found for semen samples classified as having normal morphology (sperm deformities were not described in this study). In support of this finding, Lackner et al. have shown that leukocytospermia may not necessarily have a negative impact on outcomes following assisted reproductive techniques. They reported similar fertilization rates for non-leukocytospermic samples and leukocytospermic samples (63.4% vs. 64.3%, P = not significant)[24] Corresponding pregnancy rates also did not differ significantly between the two groups.

Considering the results from these reports, it is possible that leukocytes in semen samples may have a dual effect on semen parameters. The aim of this study was therefore to evaluate the association between numerous seminal leukocytes and sperm count in Bayelsa State.

Objective and statement of the problem

A trend has been observed whereby an increasing number of patients attending the Obstetic Unit at the Federal Medical Center, Yenagoa for primary or secondary infertility complains have presented samples with leukocytospermia. Related research works on the issues of the effect of leukocytospermia on sperm count is a

divisive one. This work is borne out of the lack of such studies in Bayelsa State with the pecularity of the people lifestyle and diets.

Although extensive research has been conducted into the relationship between certain sperm parameters and leukocytospermia [25,26,27], none has been conducted in Bayelsa State. An investigation into the cause for this observation and its possible link to MAGI could provide vital insight into a condition of infertility management.

The aim of this study is therefore twofold:

1) To evaluate if a correlation exists between seminal sperm count and leukocytospermia.
2) To exert whether leukocytospermia could contribute to infertility in males.

MATERIALS AND METHODS

Study Location

This study was conducted at the departments of Microbiology and Chemical Pathology of the Federal Medical Centre, Yenagoa, Bayelsa State. Bayelsa state is located within Latitude 4^0 15^1 North and Latitude 5^0 and 23^1 South. It is also within longitude 5^0 22^1 West and 6^0 45^1 East. It is bounded by Delta State on the North, Rivers State on the East and the Atlantic Ocean on the Western and Southern parts. According to the 2006 census figures, Bayelsa has a population of about 1.7 million people.

Study subjects

One hundred (100) apparently healthy men visiting the Microbiology Department of the Federal Medical Centre, Yenagoa for semen analysis from August 2012- March 2013 constituted the study population. The study population was divided into two groups; non-leukocytospermic subjects and leukocytospermic subjects. Your sperm count is considered lower than normal if you have fewer than 15 million sperm per milliliter of semen

Ethical Clearance

Ethical approval was granted by the department involved in the study. Participation was voluntary and informed consent was obtained as verbal or written depending on the literacy level of individual participants.

Semen collection

In total, the 100 ejaculated semen samples were from two sample cohorts; patients seminal fluid without leukocytospermia (n=50) and those with leukocytospermia (n=50). As mentioned, no exclusion criteria were applied to either All samples were collected in accordance to the WHO guidelines (WHO, 2010), following a 2-3 day period of sexual abstinence. Semen was collected by means of masturbation into a sterile wide mouth plastic container, placed in an incubator (37ºC, 5% CO 2 60 minutes) prior to processing of the liquefied semen samples. There is a degree of variability amongst the donors regarding a specific time at which the samples are received, therefore, to achieve consistency in the analysis, the samples were left incubated for a set period of 60 minutes.

Standard semen analysis

The semen quality and sperm parameters were assessed in terms of the guidelines outlined by the WHO (WHO, 1999). The basic semen analysis was executed by compound microscopy and analyzed by experienced Medical Laboratory Scientists at the Microbiology Department of the Federal Medical Center, Yenagoa, Bayelsa State to ensure the most accurate results.

Sperm count

The number of spermatozoa per unit volume of semen was determined according to WHO guidelines (WHO, 1999). In order to establish the correct dilution required for each sample, an initial wet preparation on a glass slide was examined. Once the appropriate dilution of distilled water to semen has been determined according to set ratios (WHO, 2010), 10µl of the diluted sample was loaded into the counting chambers of an improved bright-light Neubauer haemocytometer (depth 100µm) (Marienfeld, Germany). Once the spermatozoa settle in the counting chamber, the appropriate haemocytometer grid for the particular dilution was examined and counted per replicate and the concentration of spermatozoa per milliliter of semen was calculated.

Statistical analysis

Data were analyzed with SPSS program (SPSS Inc., Chicago, IL, USA; Version 15) and expressed as mean ±SE. Student t-test was used for comparing values of the leukocytospermic samples and non-leukocytospermic samples. Percentages and pictorial expression were also used for data presentation.

RESULTS

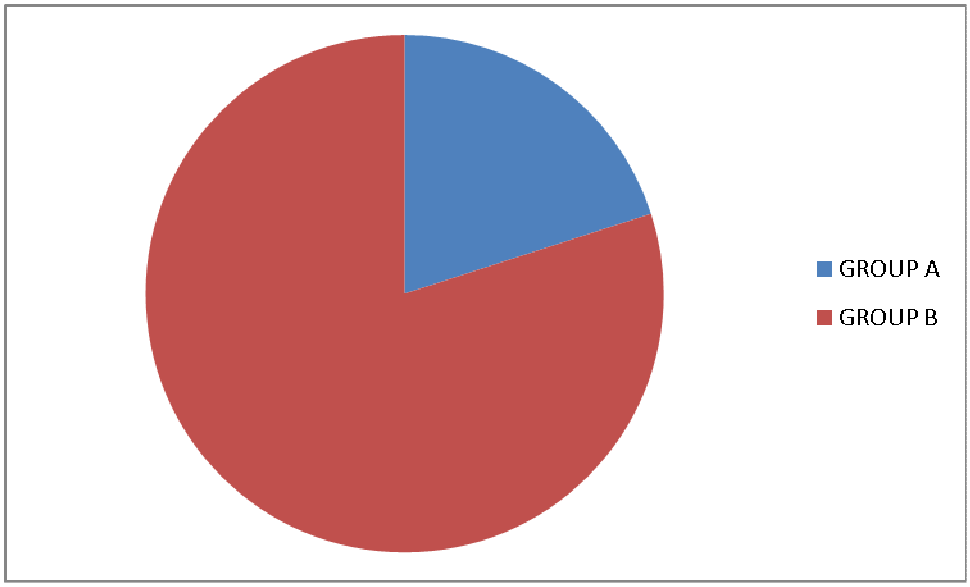
Table 1.1 shows a comparison of the Mean±SD of between non-leukocytospermic samples (10^6/ml) and leukocytospermic samples (10^6/ml)**.** Non-leukocytospermic samples sperm count **was** 47.3.0±64.8 as compared to leukocytospermic samples of 21.0±26.6. Employing computerized SPSS version, the results show that the values obtained was statistically significant (P<0.05). Hence a relationship does exist between sperm count and leukocytospermia.

TABLE 1.1: A COMPARISON OF MEAN±SD OF SPERM COUNT (10^6/ml) BETWEEN NON-LEUKOCYTOSPERMIC SAMPLES (10^6/ml) AND -LEUKOCYTOSPERMIC SAMPLES (10^6/ml)**.**

PARAMETERS MEASURE	GROUP A MEAN±SD (n=50)	GROUP B MEAN±SD (n=50)	P-VALUE	COMMENT
SPERM COUNT	47.3.0±64.8	21.0±26.6	P<0.05	S

S- SIGNIFICANT

Figure 1.1 is a Pie chart showing the percentages of Aspermic samples in Group A and B. The figure shows that that Aspermia is more common in Non-Leukocytospermic subjects as compared to Leukocytospermic subjects.



DISCUSSION

Semen is an organic fluid, also known as seminal fluid, that may contain spermatozoa.

Sperm count, or sperm concentration measures the concentration of sperm in a man's ejaculate. Over 15^7 million sperm per milliliter is considered normal, according to the WHO in 2010. This work defined leukocytospermia as a semen containing numerous pus cell upon the semen microscopy. An increased concentration of leukocytes is a molecular defense mechanism against the presence of foreign organisms and the detection of pathological concentrations of leukocytes, with the exclusion of a bladder infection or urethritis, has been suggested as a basic diagnostic tool in recognizing MAGI [8,9]. The research carried out correlating the relationship between sperm

count and leukocytospermia was statistically significant. The subjects with leukocytospermia tend to have a decreased sperm count. Also, aspermia cases where more evident in leukocytospermic subjects compared to non-leucocytospermic subjects. This effect of leukocytes on sperm cell could be attributed to high free radicals released by leukocytes, which have the capacity of reducing the quality of sperm cells by distorting its structural configuration.

The results of the current study are in agreement with those of both Aziz et al. and Ziyyat et al. mentioned previously [12,23]. In line with the results of Ziyyat et al., this study showed a decrease in sperm count with an increased leukocyte concentrations. Thus, these results can be interpreted to imply that leukocytes might have both negative effects on sperm count , with a subsequent infertility issues.

How can these contradictory effects be explained? The relationship between leukocytes and semen parameters appears to be highly complex, and may involve a number of factors, such as proinflammatory cytokines [29] and/or ROS. ROS, in particular, have featured widely in the recent literature as a possible factor in the association between leukocytes and semen parameters, and will form the focus of our discussion. Although the current study evaluated the effect of leukocyte concentration rather than ROS levels, leukocytes have been described in the literature as the primary source of ROS, and so could be viewed as a surrogate marker for ROS [12.]

As has been mentioned, the observed negative effects of leukocytospermia on semen quality have been attributed to the presence of harmful ROS [9-11]. ROS are produced by leukocytes even at leukocyte concentrations <1 × 106/mL, prompting suggestions that the leukocyte concentration used by the WHO to define leukocytospermia (>1 × 106/mL) should be lowered [11,12]. However, ROS also have a physiological function in cell signalling and have been shown to induce sperm capacitation, hyperactivation, and the acrosome reaction [28,29]. Though the production of ROS by spermatozoa has also been reported [11], it remains to be determined whether this intrinsic ROS can have a detrimental effect on semen parameters. Henkel et al. found leukocytes to correlate more with extrinsic ROS production than with intrinsic ROS production [11].

Thus, the effects of ROS appear to be twofold and, hence, any effect on spermatozoa by leukocytes could also be double-edged.

So, why would it appear that leukocytes at concentrations of >0-1 × 106/mL improve some aspects of semen quality, despite the fact that high ROS levels could be produced even at these fairly low and normal leukocyte concentrations [12]? The explanation may be that ROS have a very short half-life, and are generated constantly in the cell. However, seminal plasma has a large number of antioxidant defence mechanisms. This allows for the fact that any negative impact of ROS can be readily reduced by scavenger mechanisms [30]. Thus, the effect of ROS is dependent on the balance between oxidant and antioxidant activity, and ROS will only have negative effects once they exceed a specific threshold [10]. Thus, normal spermatozoa may have the ability to compensate for a certain concentration of leukocytes. However, the specific pathway by which leukocytes may influence morphology cannot be clarified by the findings of this study.

REFERENCES

1. Andrade-Rocha, F. T. (2005). Physical analysis of ejaculate to evaluate the secretory activity of the seminal vesicles and prostate. *Clin Chem Lab Med*; 43(11): 1203-10
2. Trum J.W., Mol B.W., Pannekoek Y., Spanjaard L., Wertheim P., Bleker O.P., Veen F van der. (1998). Value of detecting leukocytospermia in the diagnosis of genital tract infection in subfertile men. *Fertil Steril*. ;70:315–319.
3. Rodin D.M, Larone D, Goldstein M.(2003). Relationship between semen cultures, leukospermia, and semen analysis in men undergoing fertility evaluation. Fertil Steril.;79:1555–1558.
4. World Health Organization.(1999) WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. 4. Cambridge: Cambridge University Press.
5. Wolff H. (1995)The biologic significance of white blood cells in semen. Fertil Steril.;63:1143–1157.
6. Kiessling A.A., Lamparelli N., Yin H.Z., Seibel M.M., Eyre R.C. (1995)Semen leukocytes: friends or foes? Fertil Steril.;64:196–198
7. World Health Organization (2010). WHO laboratory manual for the examination and processing of human semen. Geneva, WHO Press, World Health Organization
8. Weidner, W., W. Krause, et al. (1999). Relevance of male accessory gland infection for subsequent fertility with special focus on prostatitis." Hum Reprod Update 5(5): 421-32
9. kokab, A., M. M. Akhondi, et al. (2009). Raised Inflammatory Markers in Semen from Men with Asymptomatic Chlamydial Infection. J Androl; 31(2): 114-20

10. Kaleli, S., F. Ocer, et al. (2000). Does leukocytospermia associate with poor semen parameters and sperm functions in male infertility? The role of different seminal eukocyte concentrations. Eur J Obstet Gynecol Reprod Biol 89(2): 185-91
11. Diemer, T., P. Huwe, et al. (2003). "Urogenital infection and sperm motility.mAndrologia; 35(5): 283-7
12. Aziz N., Agarwal A., Lewis-Jones I., Sharma R., Thomas A.J Jr. (2004). Novel associations between specific sperm morphological defects and leukocytospermia. Fertil Steril. ;82:621–627.
13. Gambera, L., F. Serafini, et al. (2007). "Sperm quality and pregnancy rate after COX-2 inhibitor therapy of infertile males with abacterial leukocytospermia. Hum Reprod 22(4): 1047-51
14. Sharma, R. K., A. E. Pasqualotto, et al. (2001). Relationship between seminal white blood cell counts and oxidative stress in men treated at an infertility clinic. J Androl 22(4): 575-83
15. Keck C., Gerber-Schäfer C., Clad A., Wilhelm C., Breckwoldt M.(1998) Seminal tract infections: impact on male fertility and treatment options. Hum Reprod Update;4:891–903.
16. Lackner J., Schatzl G., Horvath S., Kratzik C., Marberger M.(2006). Value of counting white blood cells (WBC) in semen samples to predict the presence of bacteria. Eur Urol;49:148–153.
17. Tomlinson M.J., White A., Barratt C.L., Bolton A.E., Cooke I.D. (1992).The removal of morphologically abnormal sperm forms by phagocytes: a positive role for seminal leukocytes? Hum Reprod. ;7:517–522.
18. Kiessling A.A., Lamparelli N., Yin H.Z., Seibel M.M., Eyre R.C.(1995). Semen leukocytes: friends or foes? Fertil Steril. ;64:196–198.[
19. Ford W.C.(2004) .Regulation of sperm function by reactive oxygen species. Hum Reprod Update. ;10:387–399.
20. Agarwal A., Saleh R.A., Bedaiwy M.A.(2003). Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril. ;79:829–843.
21. Henkel R., Kierspel E., Stalf T., Mehnert C., Menkveld R., Tinneberg H.R., Schill W.B., Kruger T.F. (2005). Effect of reactive oxygen species produced by spermatozoa andleukocytes on sperm functions in non-leukocytospermic patients. Fertil Steril.;83:635–642.
22. Sharma R.K., Pasqualotto A.E., Nelson D.R., Thomas A.J Jr, Agarwal A. (2001). Relationship between seminal white blood cell counts and oxidative stress in men treated at an infertility clinic. J Androl. ;22:575–583
23. Ziyyat A., Barraud-Lange V., Sifer C., Ducot B., Wolf J.P., Soufir J.C. (2008). Paradoxical increase of sperm motility and seminal carnitine associated with moderate leukocytospermia in infertile patients. Fertil Steril. ;90:2257–2263
24. Lackner J.E., Märk I., Sator K., Huber J., Sator M. (2008). Effect of leukocytospermia on fertilization and pregnancy rates of artificial reproductive technologies. Fertil Steril. ;90:869–871.
25. Simbini, T., E. Umapathy, et al. (1998). Study on the origin of seminal leucocytes using split ejaculate technique and the effect of leucocytospermia on sperm characteristics. Urol Int; 61(2): 95-100.
26. Omu, A. E., F. Al-Qattan, et al. (1999). Seminal immune response in infertile men with leukocytospermia: effect on antioxidant activity. Eur J Obstet Gynecol Reprod Biol ;86(2): 195-202
27. Alvarez, J. G., R. K. Sharma, et al. (2002). Increased DNA damage in sperm from leukocytospermic semen samples as determined by the sperm chromatin structure assay.Fertil Steril; 78(2): 319-29.
28. De Jonge C. (2005). Biological basis for human capacitation. Hum Reprod Update;11:205–214.
29. de Lamirande E., Jiang H., Zini A., Kodama H., Gagnon C. (1997). Reactive oxygen species and sperm physiology. Rev Reprod. ;2:48–54.
30. Saleh R.A., Agarwal A. (2002). Oxidative stress and male infertility: from research bench to clinical practice. J Androl. ;23:737–752.