

Original Research

White Blood Cell Level in Semen with Endometritis in Subfertil Couples

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Abstract

Infertility has been a severe health problem worldwide in recent decades, and various research has shown that it can be an adverse prognostic factor for infertility. 1 Ejaculation of seminal fluid into the vagina during natural intercourse may deposit spermatozoa, pollutants, and pathogens into the uterus via pelvic or penile thrusting and belling of the glans penis during coitus. In that circumstances, both women and men will be at risk of infection following natural intercourse. As a result, the association between endometritis and increased white blood cell levels in sperm and infertility symptoms such as repeated implantation failure and recurrent loss has lately arisen as a research topic. The aim of this study is to examine the relationship between endometritis seen during hysteroscopy and rising white blood cell counts in sub-fertile men's sperm. This study involved 492 infertile couples who had hysteroscopies between January and December 2021. The semen analysis was conducted at least twice over a two-week period, one from the other in accordance with WHO criteria. Hysteroscopy was conducted at the time of endometrial growth. All data analyses were conducted with IBM SPSS 23. The result showed that the mean white blood cell level in semen range difference was significant in-patient non-endometritis was 0.25 and endometritis patient was 0.66. The white blood cell level in semen correlated with the pH level and slow progressive motility in a semen specimen. This study found a correlation between women with hysteroscopy-confirmed endometritis and white blood cells in sperm, as well as a correlation between pH level and delayed progressive motility in sperm.

1. Introduction

Infertility has been a severe health concern worldwide in recent decades, and various researchers have shown that it might be a negative prognostic factor for infertility. A combination factor causes infertility.¹ Infertility can be caused by either partner or by a specific male / female spouse. Because ejaculation of seminal fluid into the vagina during natural intercourse may deposit spermatozoa, pollutants, and pathogens into the uterus via pelvic or penile thrusting and bellling of the glans penis during coitus. In that circumstance, both women and men risk infection following natural intercourse.

Male accessory gland infection is a frequent disease, with one of the characteristics as the presence of leukocytospermia with altered sperm parameters. Leukocytospermia is defined as white blood cells (WBC) in semen higher than 1 million per milliliter.^{2,3} In clinical settings, infertile men experience leukocytospermia more often than fertile men. Leukocytospermia can impede spermatogenesis and maturation while generating reactive oxygen species (ROS) that impair sperm motility, particularly progressive motility, and decreasing concentration. More common in men with leukocytic sperm than in men with normal sperm count.⁴ Leukocytospermia can impair the fertilizing capacity of sperm by interfering with the acrosomal reaction and sperm-egg fusion. Leukocytospermia has also been linked to sperm DNA fragmentation. Polymorphonuclear neutrophils are the most common form of leukocyte in sperm.^{3,5} Furthermore, the existence of several ion channels and exchange systems that control intracellular pH in sperm cell activities plays an important role.^{1,6}

Chronic endometritis is presently thought to be a chronic disease in which inflammatory cells enter the endometrial stroma. Most endometritis patients have no visible indications and the prevalence rate has been estimated at around 10%. Chronic endometritis (CE) receives little clinical attention from gynecologists and pathologists due to the time-consuming microscopic investigations, clinical symptoms, and benign nature of endometriosis. According to relevant research, chronic endometritis under hysteroscopy is characterized by microscopic polyps, interstitial edema, and patchy hyperemia. On the other hand, the association between endometritis and infertile problems, such as relapse of failure implantations or miscarriages, had recently arisen as a research topic.^{7,8,9} Endometritis can be caused by exposure to seminal fluid.¹⁰

This research aims to see the correlation of endometritis found in hysteroscopy examination

and the white blood cell level in semen in sub-fertile couples.

2. Methods

Study Population

This study involved 492 infertile couples who underwent hysteroscopy at the Bocah Indonesia Fertility Clinic between January and December 2021. Following the preliminary screening, 104 couples were enrolled in the research. Inclusion criteria: (1) having a normal sexual life with a period of intercourse 2-3 times per week; (2) the couple had no major diseases and active infectious diseases; (3) couples that never used antibiotic treatment prior to hysteroscopy; (4) semen analysis was performed within the last six months; (5) male partner had at least two semen analyses according to WHO 2010 and 2021 reference values. Exclusion criteria: (1) the patient has an endocrine problem or another disease that causes infertility; (2) the patient has had an intrauterine operation or procedure in the last two months in which ultrasonography revealed uterine malformation, fibroids, intrauterine adhesion, and tubal effusion; (3) the couple has taken birth control pills or other hormonal drugs in the last three months; and (4) the male partner has severe sperm abnormalities that cause infertility, such as cystic fibro. This study was authorized by the Ethical Committee of Primaya Hospital Tangerang, and each couple supplied written informed consent.

At least twice, separated by a minimum of two weeks, the sperm analysis was conducted. The male partner collected sperm samples after 2–4 days of sexual abstinence accompanied by masturbation without the use of any substances that could compromise sperm quality. Specimens of sperm were liquefied at 37 °C and then analyzed in the laboratory. After liquefaction, a pH test (pH strip test, MERCK) and a peroxidase test were performed on the WBCs. On samples, the total count, concentration, total motility, progressive motility, and concentration of round sperm cells were evaluated as part of standard inspection.

During the endometrial growth phase, a hysteroscopy was done. The German company TEKNO provided the Xenon cold light source, the TV camera, and the monitoring system. The hysteroscopy process utilizes TEKNO Company's intricate hysteroscopy technique. The distention media employed was physiological saline. The starting distention pressure was established between 60 and 80 mmHg, and it was altered during the procedure. All surgical procedures were carried out by the same set of surgeons, who had a clear field of vision and paid close attention to

intimal changes. During hysteroscopy, each patient's data were recorded and preserved.

Statistics Analysis

IBM SPSS 23 was used for statistical analysis. For normality testing, all data were subjected to the Kolmogorov-Smirnov test. Continuous variables that are not normally distributed are tested with The Mann-Whitney test. $P < .05$ was considered statistically significant. The Spearman test was used to determine the degree of correlation between variables. Logistic regression was performed to examine the relationship between WBC levels in sperm and endometritis in infertile couples.

3. Result

492 Infertility couples who underwent hysteroscopy in the Fertility Clinic at Bocah, Indonesia, from January 2021 to December 2021, were collected. After excluding criteria, the remaining samples were 104 infertility couples. The result was abnormal distribution (sig <0.05) with Kolmogorov-Smirnov. The age range was 25-47 years, 36.1 ± 5.55 years, and had not conceived within 12 months without contraception or after escaping their contraception uses. The result from the graphic box showed that the white blood cell range in semen specimens from endometritis female patients was higher than non-endometritis female patients.

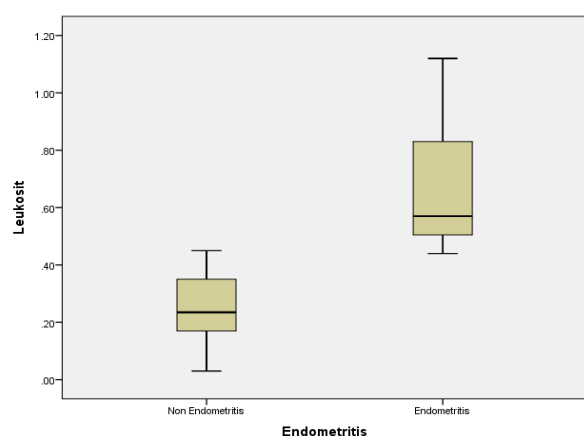


Figure 1. Graphics Scatter Endometritis and Non Endometritis

Variable	Groups	Mean (range)	Sig
White Blood Cell in Semen	Non-Endometritis	0.25 (0.03-0.45)	0.000
	Endometritis	0.66 (0.44-1.12)	

Table 1. Comparative Test

Through the Mann-Whitney test, the mean white blood cell level range in semen was significantly different ($p = 0.000$ ($p < 0.05$)), with patient non-endometritis result of 0.25 and endometritis patient 0.66. A correlation test found that higher white blood cell level in semen on the male partner was correlated with endometritis on the female partner.

The white blood cell in semen number correlated with the result of the pH level in semen specimen with Spearman test p values 0.000 ($p < 0.05$). The result from the scatter diagram showed a positive trend, confirming the correlation.

The correlation test between white blood cells and motility in semen specimens showed significant results p values = 0.025 with slow progressive (SP) motility. A positive trend in the scatter box diagram supported this result. However, RP (rapid progressive) and Non-Progressive were non-significant.

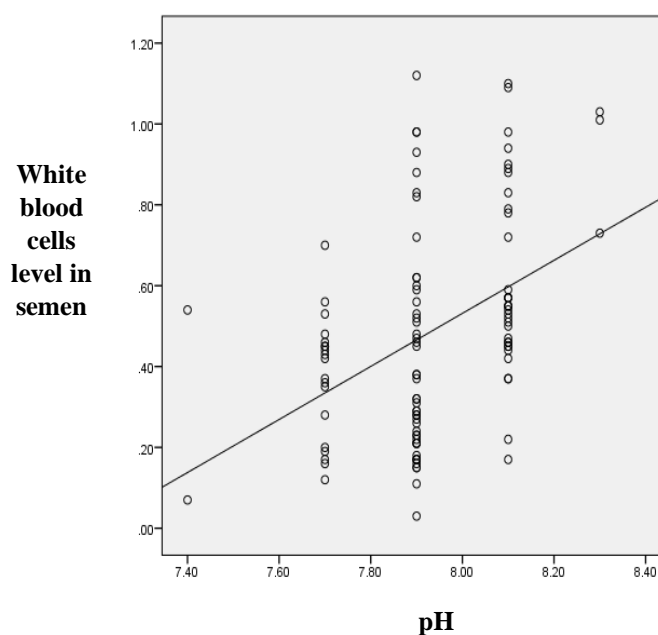


Figure 2. Graphics Scatter White Blood Cell Level in Semen with pH

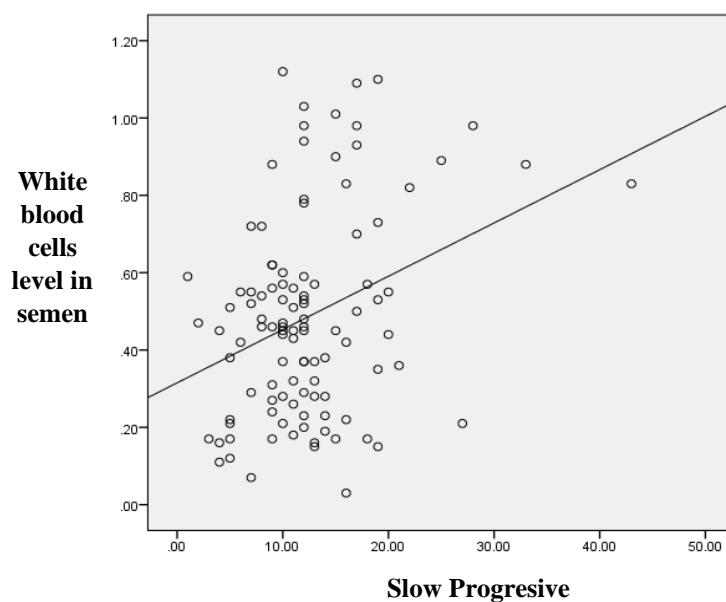


Figure 3. Graphics Scatter Box 3 White Blood Cell Level In Semen With Motility Slow Progressive

Table 2. Correlation Leucocyte with pH and Motility

Variable		p Value	Correlation coefficient	Strength
White blood Cells Level in Semen	pH	0.000	0.433	Strongly significant
	Motility			
	Rapid Progressive	0.988	-0.001	Non Significant
	Slow Progressive	0.025	0.218	Weak significant
	Non Progressive	0.974	0.006	Non Significant

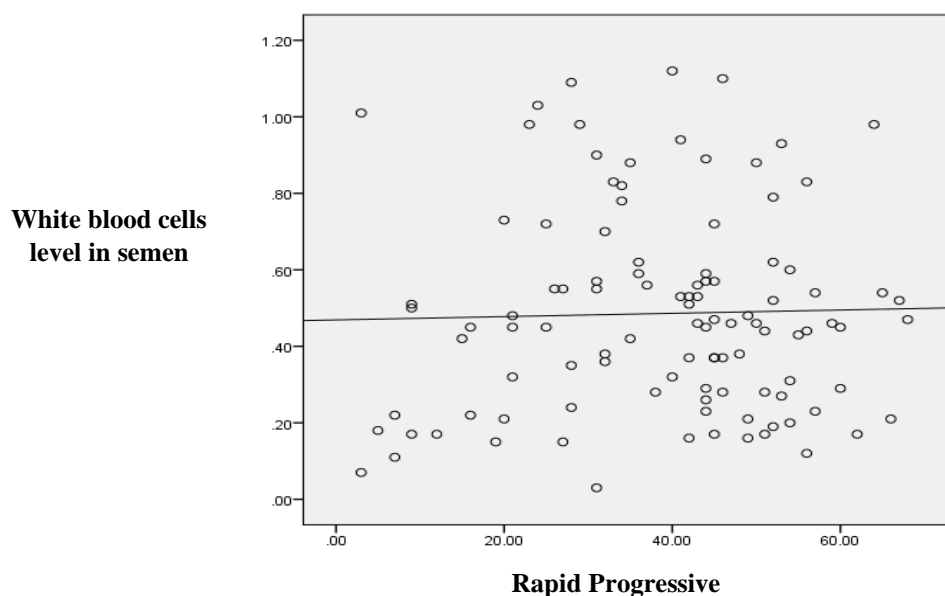


Figure 4. Graphics Scatter Box 4 White Blood Cell Level in Semen with Rapid Progressive

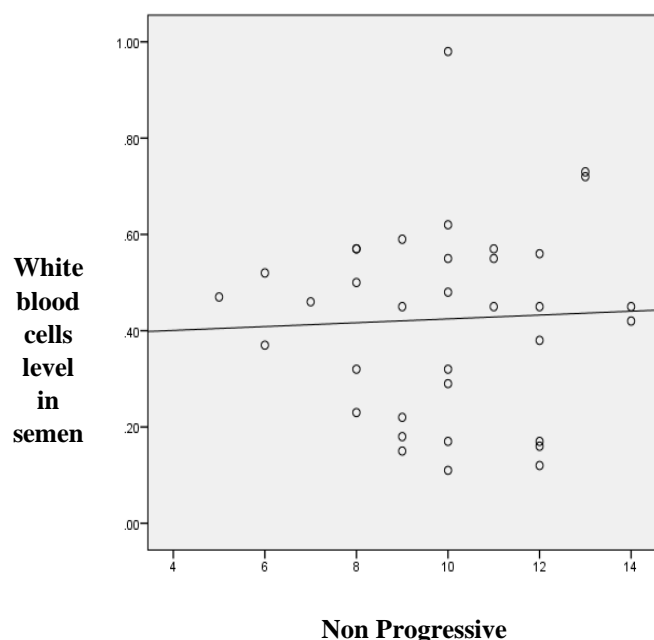


Figure 5. Graphics Scatter Box 5 White Blood Cell Level in Semen with Non Progressive

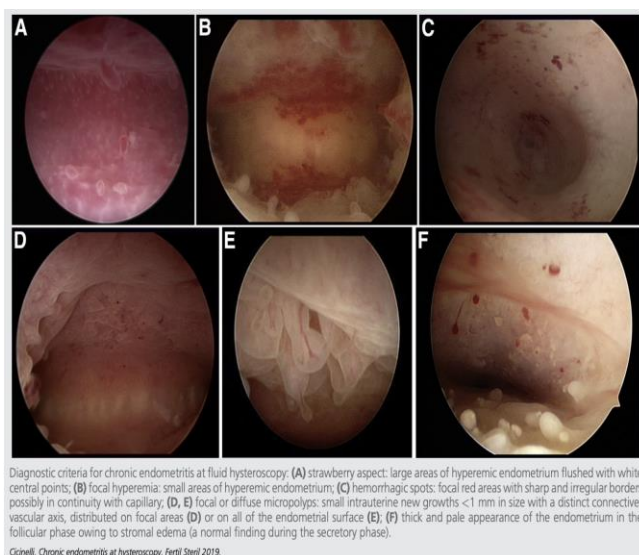


Figure 6. Endometritis in Hysteroscopy Fluid⁹

4. Discussion

In this study, we have assessed and reported that the white blood cell level in semen > 0.6 is related to endometritis in sub fertile couples. It is important to be aware that higher white blood cell level in semen specimen could cause: (1) Inhibits spermatogenesis and sperm maturation by changing cytokine levels and impairing function of Sertoli cells; (2) the most common type of leukocytes in semen, polymorphonuclear neutrophils, produce reactive oxygen species (ROS) that can impair the motility of sperm.^{1,8,11} Based on Wang et al., there was no evidence

supporting a detrimental effect of ROS on sperm, but Salvoni et al. had evidenced that increasing ROS activity and elevated tyrosine nitration had been contributory to the pathogenesis of idiopathic motility. Weinberg et al. had approved that increasing Nitric oxide from the addition of sodium nitroprusside inhibited sperm motility and correlated with NO-mediated inhibition of sperm cellular respiration.¹² Terato astheno zoospermia and necrozoospermia commonly leukocytospermia men than normospermia's.^{13,14} As long as interfering with the fusion of sperm and egg and acrosome response, leukocytospermia may impair spermatozoa's ability to fertilize. High levels of ROS and interferon- γ are produced by semen leukocytes, which inhibit the function of sperm and can reduce in vitro fertilization rates. Therefore, leukocytes in seminal plasma are an important prognostic factor for IVF and embryo transfer (IVF-ET) failure. Wolff and Anderson presented some data on the relationship between leukocytes and unexplained infertility in men. Their data shows that the samples of 51 fertile and 17 infertile men found that the infertile group had higher mean and median white blood cell counts. They also found that a more precise identification of leukocytes in sperm benefits male infertility treatment significantly, particularly regarding IVF success (IVF).^{2,5,15} According to Abhiseka and Ahmar, molecular, electrophysiological, and pharmacological research have revealed the existence of several ion channels and system of exchangers that control pH of intracellular and activities of spermatozoa. Recent studies have also shown that pH may be involved in regulating sperm fertility and that changes in pH impair sperm function.

Endometrial receptivity remains a "black box" in assisted reproductive medicine despite recent advancements. Immune cells and cytokines precisely regulate the phases of the implantation procedure. According to current research, chronic endometritis may have an adverse effect on implantation by affecting decidualization and modifying the expression of proteins involved in uterine receptivity (such as cytokines, growth factors and apoptotic proteins).^{12,16}

The ejaculation of sperm into the vagina during natural intercourse has the potential to deposit sperm, debris, and bacteria in the uterus as a result of pelvic or penile thrusts and glans penis. As a consequence of this, natural and artificial forms of insemination are the most common ways to contaminate the uterine cavity. Inflammation of the endometrium is a natural consequence of mating and is caused by the activation of cytokines and complement. Before the conceptus arrives in

the uterus approximately five days after ovulation, inflammation clears the uterus of any contaminants, seminal plasma, and additional spermatozoa that may be present. Within twenty-four to forty-eight hours of a sexual encounter, uterine contractions play a role in the evacuation of fluid and inflammatory cells. On the other hand, some females retain fluid and neutrophils for prolonged periods of time, which impairs the function of the ciliary. Alterations in the activity of the mucociliary system make it possible for infectious agents to attach themselves to the endometrium and for inflammatory cells to be discharged. Vascular degeneration causes a decrease in the return of blood from the veins to the capillary beds and disrupts the supply of hormones to the endometrium. In addition, hysteroscopy and sperm analysis have the potential to be reliable methods for identifying the cause of infertility in subfertile couples.

5. Conclusion

This study revealed a correlation between women with hysteroscopy-confirmed endometritis and white blood cells in sperm samples. The study revealed an association between the number of leukocytes and the pH level and delayed progressive motility of sperm. Due to the absence of high-quality data in the published literature, however, such a theory must remain hypothetical, needing a large-scale investigation..

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Authors' Contributions

All authors have contributed to the final manuscript. The first author contribute as follow: collected the data, drafted the manuscript, and designed the figures. The second author and third as follow: devised the main conceptual ideas and critical revision of the research. All authors discussed the result and contributed to the final manuscript.

Conflict of Interest

There were no reported potential conflicts of interest related to this article.

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