Introduction
Measurement of leukocytes in semen has been a standard component of the semen analysis, but its true significance is still unknown. The issue of whether or not leukocytospermia is detrimental to fertility is unsettled. Some studies found no detrimental effects of leukocytospermia [1, 2] but several others correlated seminal leukocytes with impaired semen parameters, especially sperm morphology and motility [3-5]. Adding to the confusion is an older study that suggested that seminal leukocytes at concentrations between 1 and 3 million/mL (M/ml) are beneficial for sperm function, arguably due to effects of cytokines or scavenging of abnormal sperm [6].

Leukocytospermia is thought to have multifactorial origin. In addition to genital tract infections, other etiologies such as smoking, alcohol consumption, and marijuana use [7] increase WBC in semen. Prolonged abstinence and certain sexual practices (use of vaginal products or anal intercourse) can produce leukocytospermia. Increased WBC in semen can be noticed in men with abnormal spermatogenesis as protective mechanism for the removal of defective sperm from the ejaculate. Finally, varicocele or vasovasostomy can result in high number of leukocytes in semen [8].

Though associating the presence of seminal leukocytes with genital tract infection would be a logical assumption, many studies fail to reveal any correlation between leukocytospermia and the presence of bacteria in the semen [1, 9, 10]. In contrast, Punab et al found that leukocytospermia correlated significantly with bacterial counts and the threshold value for leukocyte count was 0.2 M/ml [11]. This threshold is lower than the traditional World Health Organization (WHO) definition of leukocytospermia, defined as the presence of greater than 1 million leukocytes per milliliter (1 M/ml) [12]. Additional findings from other studies have also challenged the traditional threshold value for leukocytospermia [5]. It appears that any level of leukocytes in the semen between 0.2 to 1 M/ml can be associated with pathological increase of seminal reactive oxygen species (ROS) and even low levels of seminal leukocytes are associated with sperm DNA damage [13, 14].

The decision to treat infertile patients with the newly suggested cut off value for leukocytospermia depends on identification of the cause, the prognosis and the natural history of leukocytospermia. To our knowledge, there has been only one prospective study that examined the natural history of leukocytospermia; however this study used the traditional threshold of 1M/ml [15]. Our study has focused on monitoring the trend of seminal leukocytes levels over time in infertile patients with leukocytes at or above 0.2M/ml.

Materials and Methods
Following approval from the Institutional Review Board, medical records of men presenting to the Cleveland Clinic Male Infertility Center for a fertility evaluation during the period from September 2006 to January 2008 were retrospectively reviewed. Analysis with Endtz tests was performed only on patients with no prior treatment. A subset of men was identified to have leukocytospermia as defined by a level of or more than 0.2 million white blood cells per milliliter of semen (M/ml) and would have
subsequent assessment of seminal leukocytes at a minimum of 21 days interval or more. Semen samples were collected by masturbation in a sterile wide-mouthed calibrated container after an abstinence period of 2-5 days. Leukocytospermia was assessed first by calculating the number of round cells per high power field. Endtz test was done if rounds cells were more than 1-2 per high power field. Endtz test is used to differentiate leukocytes from immature sperm cells and leukocytospermia is diagnosed when WBC exceeds 0.2 M/mL [16].

Changes in the levels of seminal leukocytes were recorded over several time periods after initial evaluation. Differences in semen leukocyte levels across all time periods were tested with the Kruskal Wallis rank test. Differences between individual time periods were evaluated with the paired t-test. The rate of spontaneous resolution was calculated, defining resolution as decrease to less than 0.2M leukocytes per mL. The sequential increases or decreases in leukocyte levels over time were also calculated. A p value < 0.05 was considered statistically significant and calculated by using the statistical software, S-plus 7.0 (Insightful, WA).

Results
A total of 33 infertile patients had leukocytospermia based on the newly set cut off value. 81 different Endtz tests were performed (range 2-5 per patient) prior to giving any treatment. This allowed for 48 intervals among patients to compare increase or decrease in semen leukocyte level. The average time between Endtz tests was 151 days (range 21-1421). The average patient age was 35.2 years (range 23-48); 22/33 (67%) patients presented with primary infertility and 11/33 (33%) presented with secondary infertility. Eleven patients had a unilateral grade 1 or 2 left varicocele, and one had bilateral varicoceles (grade 1 on the right and grade 2 on the left).

Figure 1 shows the trend in semen leukocyte levels over time for those patients who presented with a level of leukocytospermia at or above 0.2M/mL or greater. Patients who had an initial leukocyte level of zero based on Endtz test are also included because on subsequent measurements they were discovered to have leukocytospermia on at least one of these semen analyses. There is no clear directional trend for leukocytospermia over time in patients with elevated semen leukocyte levels who did not receive any intervention [Figure 1].

When consecutive Endtz tests were analyzed as individual incremental changes, the following was revealed. There were 23 increases and 17 decreases in absolute leukocyte concentration. There were eight instances where consecutive Endtz values were unchanged. In only seven of 33 patients did the semen leukocyte levels fall below 0.2M/ml, resulting in a spontaneous resolution rate of 21% and in three of these, leukocytospermia returned spontaneously.

Discussion
The impact of seminal leukocytes on male infertility and their etiology is still a subject of considerable debate. Rodin et al found no correlation between leukocytospermia and presence of bacteria on semen culture nor did leukocytospermia correlate with semen parameters in their study [2]. Lackner et al showed that leukocyte count between 0 to 1 M/mL is correlated positively with sperm parameters. However; deterioration in semen parameters would be observed if leukocyte count exceeds 1 M/mL [17].

There is much controversy regarding the role of leukocytes in semen. Some claim that leukocytes may not be just a response to bacterial infection but rather act to scavenge abnormal germ cells. This idea was first brought up by Kiiessling in 1995 and discussed again when Kaledi et al found that sperm from semen samples with leukocyte concentrations between 1 and 3 M/ml performed better on certain measures of sperm function, such as the acrosome reaction and hypo-osmotic swelling test [6, 18].
Others have found that leukocytospermia is inversely associated with motility and percentage of normal forms [3, 4]. Gdoura et al correlated between leukocyte counts in semen and bacterial pathogens in seminal samples of infertile men to establish the minimum leukocyte count associated with significant bacteriospermia in 116 patients. The sensitivity/specificity for detecting bacteria at a cut-off level of $\geq 1 \times 10^6$ leukocytes per ml was found 20.3%/81.5%. In semen samples with a cut-off level of $\geq 0.275 \times 10^6$ leukocytes per ml showed the highest sensitivity/specificity ratio. A significant correlation was found between bacteriospermia and leukocytospermia at the cut-off level of $\geq 0.275 \times 10^6$ leukocytes per ml of semen samples. They thus proposed that this is a possible new cut-off level to predict the presence of bacteria in semen of infertile men [19].

Thomas et al found that even with a threshold of 0.5M polymorphonucleocytes (PMN) /ml measured by the Endtz test, sperm morphology was negatively impacted by the presence of leukocytospermia [5]. The impact of leukocytes on fertility outcomes is uncertain, so is the correlation of leukocytospermia with actual bacterial infection of the genital ducts. The high level of skin contamination and difficulty obtaining an accurate specimen only confounds the situation. Many studies show no association between the presence of seminal leukocytes and bacteriospermia [1, 9, 10]. Others have suggested that seminal leukocyte count correlates significantly with the number of different microbes as well as the total microbial count [11]. Using a receiver operating curve (ROC), the authors found the traditional threshold for leukocytospermia of greater than 1M/ml to have low sensitivity. A threshold value of 0.2M/ml had a better sensitivity/specificity ratio.

The total number of peroxidase -positive cells in the ejaculate may reflect the severity of an inflammatory condition [20]. There is debate over whether the World Health Organization (WHO)-suggested concentration of $10^6$ leukocytes per mL in semen is a valid threshold value for leukocytospermia. Reports of cut-off values for peroxidase-positive cells in fertile men vary from $1 \times 10^6$ to $2 \times 10^6$ total leukocytes per ml [20]. Previous editions of WHO manual have taken $1 \times 10^6$ leukocytes per ml as the threshold for leukocytospermia. On the basis of the outcomes of semen quality and results of in-vitro fertilization, some consider the threshold as very low [20], and some consider it as a very high cut off [11, 14, 21]. Yet, the factors on which the effect of leukocytes depends are impossible to conclude from a semen sample, for example; timing and anatomical location of the infiltration, nature of the leukocytes involved and activity [22-24]. The new WHO manual [21] provides a method for assessing even lower sperm concentrations than $1 \times 10^6$ per mL precisely, and even larger volumes (by using more or larger chambers) could be assessed if $1 \times 10^6$ round cells per mL really needs to be assessed precisely [25].

In addition to the evidence for leukocytospermia, at levels less than 1M/ml, being associated with microbial counts and poorer sperm morphology, the WHO definition of leukocytospermia has been challenged by recent studies which associate elevated oxidative stress with even low levels of seminal leukocytes.

Sharma et al noticed increased oxidative stress in semen samples containing even low level of leukocytospermia compared to samples with no seminal leukocytes [14]. Other studies confirmed leukocytes, along with immature germ cells, as the major source of ROS in human semen [26]. Basically, the cellular mechanisms for the generation of ROS in leukocytes and spermatozoa are the same, yet in leukocytes it is a physiological necessity to release large amounts of superoxide into phagocytic vesicles during the killing action of pathogens [27]. Sperm DNA damage has also been associated with leukocytospermia [28]. Henkel et al showed that there was significantly higher ROS levels and DNA damage at seminal leukocyte concentrations greater than 0.1M/ml compared to samples with less than 0.1M/ml [13].

The important questions now is, what are the proper treatments for infertile men who have leukocytospermia? Since leukocytospermia can be attributed to different reasons, the most suitable answer would be to first treat the cause. Clinical assessment supplemented by proper microbiological work up is needed to disclose the nature of leukocytospermia. Environmental culprits such as smoking, alcohol and marijuana should be discontinued. In cases of accessory gland infection such as prostatitis, a study was performed on one hundred two men with leukocytospermia identified by using Bryan-Leishman stain. These patients were given antibiotic treatment and observed for resolution of leukocytospermia. Patients were divided into four groups according to the treatment...
they received as follows: treatment groups with no treatment; antibiotic treatment alone; frequent ejaculation alone; and antibiotic treatment with frequent ejaculation. These groups were compared for resolution of leukocytospermia. It was observed that significant resolution of leukocytospermia occurred in all treatment groups at 1 month compared with no treatment [29]. Antibiotic treatment, frequent ejaculation, and antibiotic treatment with frequent ejaculation effectively treat leukocytospermia immediately after the treatment phase. However, on the long run patients who received antibiotic treatment coupled with frequent ejaculation achieved better resolution of infection after three months of intervention.

It remains to be established if empiric treatment with antibiotics and antioxidants will be indicated and justified in patients with low level leukocytospermia. Empiric antibiotic therapy has its disadvantages such as emergence of resistant bacteria and toxic effects on sperm function. It is first imperative to define the natural history of leukocytospermia in this context. Lackner et al were the first to address this issue in their recent study. They reported that 43% of the time leukocytospermia resolved without treatment [15]. This figure is quite a bit higher than the 21% resolution rate encountered in our study. The explanation for this difference lies most likely in the cut-off value for the definition of leukocytospermia. In Lackner study, 43% of the time the level of seminal leukocytes decreased to below the WHO threshold of 1 M/ml. It is not known what percentage of the time the level of seminal leukocytes decreased to below 0.2M/ml. If one is considering levels below 1M/ml as possibly deleterious, it is important to know what percentage of cases decreased below a newer, lower threshold value. In our study we found that in seven of 33 patients (21%) leukocytospermia decreased to below 0.2M/ml and in three of these seven (42%), it returned back spontaneously.

When semen leukocyte levels (based on the newly suggested cut off value) were plotted over time there was no clear trend upwards or downwards. This can be expected in a situation with low level of spontaneous variation. When defined by a lower threshold value, leukocytospermia seems to persist over time, or at least does not resolve spontaneously. This information can now be used to assess treatment outcomes of low level leukocytospermia. The selected treatment that reduces seminal leukocyte level below 0.2M/ml in more than 21% of the time is considered to be effective.

Proper antibiotic regimens for genital tract infections have been found to improve semen parameters with appropriate reduction in seminal WBC count and ROS production. In addition to the positive effects of antibiotic treatment on sperm output, spontaneous pregnancy rates (40%) have also been observed especially in infertile men with prostatitis and prostatovesiculitis. At 3 months after therapy discontinuation some sperm parameters, such as seminal WBC concentration and ROS generation, were improved in patients with prostatitis (PR) and prostatovesiculitis (PV); whereas no improvement was seen in patients with prostatovesicoepididymitis. [30]. It is also reported that the combined use of immune modulators and anti-oxidants helps protect the sperm during maturation and migration and gives improved sperm function[31].
Several limitations to this study require mention. First, the sample size is small and the findings may not extrapolate to larger populations. Second, the study population was from a single institution and geographical region which may not represent all groups of patients with leukocytospermia. The etiology of leukocytospermia may vary with incidences of various causative agents in different regions.

Also, we calculate a 21% spontaneous resolution rate, but three of the seven patients in whom leukocytospermia had resolved, experienced recurrent leukocytes in the semen prior to any intervention. Therefore, the true resolution rate may be lower but the number of patients in whom leukocytospermia resolved was too small to draw any conclusions based on this matter.

In conclusion, the true threshold value for treating significant leukocytospermia remains to be established. If low level leukocytospermia proves to be detrimental, as some data indicates, the natural resolution rate needs to be defined. The spontaneous resolution rate of 21% that we observed serves as background data against which the efficacy of future treatments can be compared. Larger prospective trials are critical to characterize the role of lower levels of leukocytospermia on fertility.

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References