Abstract
The objective of this study is to detect the leukocytes concentration in the semen of infertile and fertile patients. The study include three hundred and forty men refered to IVF Institute, Baghdad Teaching Hospital,. They are 290 infertile men and 50 fertile men (control). Semen analysis was done and the patients divided into subgroups according to male infertility factors. Seminal myeloperoxidase cytochemical test (Endtz method) performed for all specimens to differentiate between the leukocytes and other round cells. The concentration of Leukocytes were account using high power field (HPF) method and Endtz.

The results revealed Leukocyte concentration in normospermic patients (control) by using HPF method was significantly (P<0.01) higher than Endtz method. In oligospermic, asthenospermic and oligo-asthenoterato spermic men, the mean of leukocyte concentration by using HPF was significantly lower (P<0.05) compared to using Endtz method. There was a highly significant (P<0.01) decrease in the number of leukocytes by using Endtz method compared with the round cells detected by using HPF method.

It was concluded that myeloperoxidase (Endtz) method can be detect the leukocytes concentration in the semen and differentiate them from other round cells. This method is easy to performed, complete with short time with high accuracy, not cost and can sustain the correct treatment.

Introduction
The presence of leukocytes in the semen has been associated with poor semen quality and positive correlation between leukocytospermia and abnormal sperm morphology [1,2]. Results of sperm penetration assay, a predictor of male infertility, have been shown to have a
negative correlation with the number of leukocytes in human semen [3]. It has been reported that the presence of White blood cells (WBCs) can compromise the fertilizing potential of sperms in the zona-free hamster ova penetration tests [4]. However, Other workers found no association between conventional semen parameters and leukocyte concentration in human semen. But the presence of immature sperm cells is associated with decrease fertilizing capacity in sperms in vitro [5, 6]. Thus, in fertile individuals, round cells usually represent less than 5% (≤5 cells/HPF) of the total cellular content of semen [7]. Round cells in semen can be distinguished into; a) White blood cells b) germinal cells and others. Differentiation between these cell types is considered of utmost importance for diagnostic and therapeutic purposes [8].

Unfortunately, the routine assessment of semen samples in most Iraqi laboratories does not have an accurate method(s) to detect the leukocytes. Therefore, the objective of this study is to differentiate between WBCs and other round cells using myeloperoxidase cytochemical staining method for the patient’s semen that been in our IVF Institute.

**Materials and Methods**

**Semen Samples**

The study was carried out in the Institute of Embryo Research and Infertility Treatment (IVF Institute), University of Baghdad through December, 2001 to November, 2003 includes three hundred forty of infertile and fertile Iraqi patients, their ages ranged between 20-55 years. Complete history is reported. The clinical examination performed by a consultant urologist in charge of male infertility in the Institute with Doppler ultrasound to detect any abnormalities.

**Seminal Fluid Analysis**

Seminal fluid samples were collected after 3 to 7 days of abstinence, directly into a clean dry and sterile disposable wide mouth container by masturbation in especially allocated room for this purpose adjacent to the laboratory. Immediately afterwards, all semen samples were placed in an incubator at 37°C till complete semen liquefaction. The samples then assessed to macroscopic and microscopic examination as described by WHO [7].

**Myeloperoxidase Cytochemical Test**

This test is performed on suspended cells in liquefied semen specimen and quantitated by counting stained cells [9]. A 20 μL of liquefied semen specimen in a micro-centrifuge tube added to 20 μL of sperm preparation medium and 40 μL of working benzidine solution. Following the mixing at room temperature, Neubaur counting chamber loaded with 5 μL of the prepared solution. Under 20 X bright field objective lens, all leukocytes are stained dark brown in color with round shape. The cells are counted in all RBCs fields, which equal to one mm³. Number of WBCs calculated by multiplying total number of cells by 4 to correct for dilution factor. The total WBCs number will be X10⁷/ml semen. This number corrected to million/ml by dividing by 10[19].

The results are analyzed using student’s t-test, considering p-value of 0.05 as a statistically significant difference [11].

**Results**

Table-1 showed the comparison between two methods of leukocyte count to detect the leukocyte concentration in the semen of male infertility factor groups. The mean of leukocyte concentration in normospermic patients (control) by using HPF method (7.25 m/ml) is significantly (P<0.01) higher compared with Endtz (0.97 m/ml) method. In all male infertility groups namely: oligospermia, asthenospermia, astheno-teratospermic, teratospermia and oligo-astheno-terato spermic men, the number of leukocyte by using HPF method is 15.3 m/ml, 14.9 m/ml, 17.5 m/ml, 21 m/ml and 14
The mean of leukocyte concentration in those patients is significantly lower ($P<0.05$) by using Endtz methods (Table 1).

Comparison between HPF and Endtz methods of patients ($n=65$) complaining from infertility with more than $5 \text{ m/ml}$ round cells is shown in Figure 1. There is a highly significant ($P<0.01$) decrease in the number of leukocytes by using Endtz method ($0.8 \text{ m/ml}$) compared with the round cells detected by using HPF method ($7 \text{ m/ml}$).

**Discussion**

The study revealed that the number of estimated leukocytes (round cell) by using HPF method was higher than Endtz method. This because using HPF can't distinguish between the WBCs and other round cells found in the semen namely; spermatocytes, spermatid, epithelial cells, prostatic cell [7]. Wolff, et al. [12] noticed that the presence and number of WBC in the ejaculate commonly reported on routine semen analysis. However, under ordinary light microscopy i.e. HPF method, WBC can not be differentiated from immature sperm precursors. Therefore, laboratory reports depending only on microscopic examination that indicate the presence of WBC are only reporting round cells that, in reality, may not contribute to a pathologic process. Unfortunately, most of the governmental and private laboratories in Iraq used HPF method to detect the leukocytes as pus cells in the routine seminal fluid analysis.

On the other hand, there are 65 out of 290 patients considered leukocytospermic depending on HPF method results. All of them were treated with different antibiotics therapy for at least two months (data from case history sheet) with no response. Following the performance of Endtz method for their semen, the number of leukocyte is less than one m/ml ($0.8 \text{ m/ml}$) which is normal value as shown in Figure (1). It has been reported that treatment with antibiotic may have negative effect and correlation on certain sperm function parameters e.g. sperm concentration, sperm motility and sperm morphology [13]. It is possible to avoid the bias treatment for such patients by performing or ordering semen culture [14] and emphasized the growth of microorganisms. Nevertheless, detecting the leukocytes in the semen at the time of semen fluid examination using myeloperoxidase method may be the proper first step recommended to prevent the random treatment and negative effect of antibiotics. Moreover, it is also important to recheck the semen culture after therapy to ensure that treatment has been adequate [15].

Researchers have shown that increased levels of WBC in the ejaculate commonly are found in infertile men, when compared with fertile controls [16]. Whereas, Curtis [17] found that many of bacteria isolated from infertile and fertile men have considered to be part of normal flora, but some organisms were probably related to infertility.

The results of male infertility groups revealed high concentration of leukocytes in the semen of astheno-teratospermic and teratospermic men. Leukocytospermia cause alterations in the spermatogenic events result in the release of immature, abnormal spermatozoa in the ejaculate. Immature spermatozoa display a high content of DNA damage, alteration in protamination, chromatin packaging and excessive reactive oxygen species production [18].

The most reliable method for the differentiation of round cells in semen is the peroxidase cytochemistry with the use of benzidine. The distinction between WBC, and immature germ cells is made possible because Endtz solution turn brown intracellularly due to the effect of peroxidase or hydrogen peroxide [19]. Thus, these cytochemical method can staining the leukocytes e.g. neutrophils, macrophages and polymorphonuclear neutrophil (PMN). It is concluded from
this study that myeloperoxidase (Endtz) method can be utilized to detect the leukocytes in the semen and differentiate them from other round cells. Estimation of leukocytes as pus cells in the semen by HPF is completely not recommended, leading to incorrect and bias treatment. Myeloperoxidase cytochemical test is easy to performed, complete with short time with high accuracy, not cost and can sustain the correct treatment. It can be used in any andrology laboratory in Iraq.

References

Table 1 Comparison between methods to detect the Leukocyte concentration in the semen of male infertility factor groups.

<table>
<thead>
<tr>
<th>Male infertility factor groups</th>
<th>Methods of Leukocyte count</th>
<th>N</th>
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<tbody>
<tr>
<td>HPF *</td>
<td>Endtz +</td>
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<tr>
<td>Normospermia(control)</td>
<td>7.25±0.79</td>
<td>50</td>
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<tr>
<td>Azoospermia</td>
<td>7.7±1.1</td>
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<tr>
<td>Oligospermia</td>
<td>15.3±1.8</td>
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<td>Asthenospermia</td>
<td>14.9±2.2</td>
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<tr>
<td>Astheno - teratospermia</td>
<td>17.5±2.6</td>
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<tr>
<td>Teratospermia</td>
<td>21 ± 3.9</td>
<td>55</td>
</tr>
<tr>
<td>oligoasthenoteratospermia</td>
<td>14.0±1.3</td>
<td>67</td>
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</table>

Values are mean ±SEM
* P > 0.01 significantly different from HPF
HPF = High Power Field
Methods of Leukocyte Detection

Figure 1: Comparison between HPF and myeloperoxidase (Endtz) methods of infertile patients with More than $5 \times 10^6$ Round cells /ml semen

$n = 65$

Student's t-test

* $P > 0.01$ significantly different from other method.

HPF = High Power Field