A Comparison of Makler Counting Chamber and Improved Neubauer Hemocytometer in Sperm Concentration Measurement

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Semen analysis, especially sperm counts, is still the most commonly used routine procedure in male fertility diagnosis. Although the conventional methods have long been standardized largely through the efforts of the World Health Organization (WHO), various methods have been employed in many laboratories for determination of sperm concentration, e.g. Makler counting chamber method, Microcell counting chamber method(1-3). The WHO Manual discouraged the use of white-blood-cell pipettes for making the initial volumetric dilution of the semen and recommended that positive displacement pipettes be used(3,4). Using Makler counting chambers is popular in many laboratories in Thailand because it is very simple, rapid and more convenient. The sperm count can be made rapidly and directly from an undiluted sample by counting spermatozoa in the area of a grid located with in the cover glass. However, the WHO manual discouraged the use of Makler chamber for determining sperm concentration because the method for determining sperm concentration, which involves volumetric dilution and hemocytometry, has been shown to give reliable and accurate results(3,5). Several investigators have demonstrated that the Makler counting chamber method gives reliable and accurate results(6-8).

On the other hand, many reports have shown that the Makler chamber is an inaccurate method and the routine use of this counting chambers is not recommended(5,9). The aim of the present study was to compare the use of the Makler counting chamber with the use of the positive displacement pipette and the improved Neubauer hemocytometer according to WHO guideline in sperm concentration measurement.

MATERIAL AND METHOD

Semen samples

Fifty-five ejaculates from patients attending the Infertility clinic at Department of Obstetrics and Gynecology, Chulalongkorn Hospital were kept at 37°C for at least 30 minutes for liquefaction. Of these ejaculates, the azoospermic samples were excluded. These semen samples were manually counted in two devices : (1) a Makler counting chamber (Sefi Medical Industries, Haifa, Israel); (2) an improved Neubauer hemocytometer (American Optical Company, Buffalo, NY) according to World Health Organization guideline. The procedures for counting the spermatozoa are as follows. All of the samples were counted as described previously and the results expressed as 10⁶ spermatozoa/ml.

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Makler counting chamber method\(^{(6,7)}\)

A drop from a well-mixed specimen of semen was placed on the chamber and covered. The microscope with a x20 objective and x10 eyepiece was used. The sperm heads within the squares of the grid were counted in the same way as blood cells counted in hemocytometers. Counting sperm heads contained within a strip of 10 squares, a number describing sperm concentration in millions per milliliter was obtained.

Improved Neubauer hemocytometer method\(^{(3)}\)

A 1:20 dilution was made from each well-mixed sample by diluting 50 µl of liquefied semen with 950 µl of a diluent. The latter was prepared by adding to distilled water 50 g of sodium bicarbonate (\(\text{NaHCO}_3\)), 10 ml of 35 per cent (v/v) formalin, and making up the solution to a final volume of 1,000 ml. The dilution of samples were adjusted according to the preliminary examination of the sperm concentration. A positive displacement type of pipette (SMI Model D-50, Scientific Manufacturing Industries, Emeryville, CA, U.S.A.) was used for making volumetric dilutions of the semen.

The diluted specimen was thoroughly mixed and a drop (10-20 µl) transferred to each chamber of an improved Neubauer hemocytometer and covered with a cover glass. The hemocytometer was allowed to stand for about 5 minutes in a humid chamber to prevent drying out. The cells sedimented during this time and were then counted, under a phase-contrast microscope, at a magnification of 200 to 400 x. Only spermatozoa (morphologically mature germinal cells with tails) were counted; 'pin-heads' or tailless heads were not counted. The spermatozoas in the improved Neubauer hemocytometer chamber were counted according to WHO guideline\(^{(3)}\).

Statistical analysis

Statistical analysis was performed using mean and standard deviation and reported as the mean ± S.D. Mean sperm concentration was compared between Makler chamber method and improved Neubauer hemocytometer method using paired t-tests. All tests were performed against a two-sided alternate hypothesis, and p values < 0.0001 were considered significant. Data from Makler counting chamber were compared with those obtained by the improved Neubauer hemocytometer method by calculating and plotting the percentage of differences of paired data as suggested by Bland and Altman\(^{(10)}\). All statistical tests were performed with the Statview™ +512 (Brain Power, Inc., Calabasas, CA) software package.

RESULTS

Table 1 shows the mean, standard deviation between the groups of paired measurements of sperm concentration by both techniques.

<table>
<thead>
<tr>
<th>Classification*</th>
<th>Improved Neubauer hemocytometer method</th>
<th>Makler counting chamber method</th>
<th>Percentage differences</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>11.2 ± 4.9</td>
<td>14.5 ± 7.1</td>
<td>+32.7 ± 29.2</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>20-40</td>
<td>26.0 ± 4.7</td>
<td>30.5 ± 7.5</td>
<td>+19.1 ± 31.0</td>
<td>&lt;0.0001**</td>
</tr>
<tr>
<td>40-60</td>
<td>51.8 ± 7.1</td>
<td>55.1 ± 12.4</td>
<td>+7.7 ± 28.0</td>
<td>0.688 (NS)</td>
</tr>
<tr>
<td>60-80</td>
<td>70.6 ± 6.2</td>
<td>73.7 ± 18.1</td>
<td>+4.4 ± 21.9</td>
<td>0.613 (NS)</td>
</tr>
<tr>
<td>80-100</td>
<td>83.0 ± 3.4</td>
<td>79.0 ± 13.3</td>
<td>-4.9 ± 14.5</td>
<td>0.409 (NS)</td>
</tr>
<tr>
<td>&gt;100</td>
<td>149.2 ± 41.8</td>
<td>146.3 ± 49.6</td>
<td>-2.3 ± 15.9</td>
<td>0.690 (NS)</td>
</tr>
</tbody>
</table>

* Results were classified according to the standard procedure before statistical analyses.

** Statistical significance (p < 0.0001)

NS : No statistical significance.
Linear regression analysis of the sperm concentration obtained with Makler counting chamber and of those obtained with improved Neubauer hemocytometer is shown. (Fig. 1) Linear regression analysis gave an equation: $Y = 0.28452 + 0.97305X (R = 0.931)$, where $Y$ are the sperm concentrations obtained with improved Neubauer hemocytometer and $X$ are the sperm concentrations obtained with the Makler counting chamber. There was good correlation between sperm concentration by both techniques. (Fig. 1)

**DISCUSSION**

This study has provided clear evidence that reliable determination of sperm concentration as part of routine semen analysis requires the use of carefully standardized methods. Results were classified according to the standard procedure before statistical analyses. Both values correlated well. (Fig. 1) Sperm concentration obtained with the Makler counting chamber was not statistically different from those determined by the improved Neubauer hemocytometer in semen samples with concentrations over $40 \times 10^6$ /ml. But using the Makler counting chambers caused a shift in concentrations, which were more often overestimated significantly ($p < 0.0001$) in semen samples with concentrations less than $40 \times 10^6$ /ml. (Table 1, Fig. 2) However, the average concentration obtained using the Makler counting chamber was 11.2 per cent higher than that obtained using improved Neubauer hemocytometer. Previous study showed the result using the Makler counting chamber was 62 per cent higher$^9$.

The standard technique for sperm count involves the use of a hemocytometer. The method consists of several steps preparing 1:20 or 1:100 diluting of the original sample, thoroughly mixing the diluent, inserting the diluted sample in a special way into the chamber, and allowing it to rest for about 20 minutes to permit the precipitation of spermatozoa to the bottom of the chamber before the counting itself is performed. This method requires an experienced examiner and the use of carefully standardized methods which is more complicated. This includes all steps of the procedure, from taking the initial semen aliquot through its dilution, mixing, sampling and loading into the hemocytometer, to counting the spermatozoa on the hemocytometer grid.

The Makler chamber for sperm count and motility examination, which is only 10 μm deep, enables free horizontal movement of spermatozoa in one focal plane and provides conditioning for the examination of undiluted samples. The sperm count can be made rapidly and directly from an

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**Fig. 1** Linear regression analysis of the sperm concentration obtained with the Makler counting chamber and of those obtained with the improved Neubauer hemocytometer.
undiluted sample by counting spermatozoa in the area of a grid located within the covered glass. The chamber provides for the full movement of the spermatozoa with minimal friction between the surfaces of the chamber, so that the spermatozoal speed is practically unaffected. As a result, the semen may be analyzed without dilution, in one step and quickly. This technique is very simple and rapid. Both sperm count and motility estimation can be done in the same chamber. As sperm dilution is not necessary, no accessories such as pipettes, solutions, and facilities for pipette flushing and drying are needed. As the chamber is very thin and all spermatozoa can be seen immediately and in one focal plane, there is no need to wait for sperm to be precipitated on the bottom of the chamber, a process which takes about 20 minutes when using the hemocytometer. The Makler counting chamber can be used with most types of microscopes and with no additional equipment or preparations. Semen analysis is more reliable and easy to perform even by an inexperienced examiner. All of these advantages make this instrument a helpful tool in every laboratory where routine semen analysis is done.

Although methods exist for determining sperm concentration without dilution, they require the use of special counting chambers with a precise depth of 10 μm, so that all the spermatozoa are in the same plane of focus under the microscope. In view of these potential instrument errors, the routine use of Makler counting chambers cannot be recommended. An improved Neubauer hemocytometer should be used when taking precise sperm concentration measurement.

The degree of error in determining sperm concentrations using the Makler chamber will not usually cause significant discrepancies in clinical diagnosis in semen samples with concentrations over 40 x 10⁶/ml. Contrary to these findings, the sperm concentrations are significantly higher in semen samples with a concentration less than 40 x 10⁶/ml. Overall, these results suggest that the measurements obtained with the Makler counting chamber are inaccurate. Therefore, the Makler counting chamber method is not suitable for use in epidemiological studies, or in research on infertility diagnosis or fertility prognosis using discriminant function or multiple regression statistical procedures because accurate and reliable data are
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essential. These differences in sperm measurements emphasize the importance of method of sperm concentration measurement on data reliability. Quality control and standardization of sperm concentration measurement is necessary for every laboratory.

SUMMARY

Evaluation of male fertility is based predominantly on results from semen analysis and determination of the sperm concentration is one of the main parameters of the analysis. To assess the accuracy of sperm concentration measurements by Makler counting chamber, manual sperm counting of 55 semen samples were made using a Makler counting chamber, and compared with concentration values measured using an improved Neubauer hemocytometer according to the World Health Organization guideline (standard procedure). Results were classified according to the standard procedure before statistical analyses. Both values correlated well. Sperm concentration obtained with Makler counting chamber was not statistically different from those determined by improved Neubauer hemocytometer in semen samples with concentrations over $40 \times 10^6$ /ml. But using Makler counting chambers caused a shift concentrations, which were overestimated significantly ($p < 0.0001$) in semen samples with concentrations less than $40 \times 10^6$ /ml. Overall, Makler chamber counts were 11.2 per cent higher. Although less complicated than the improved Neubauer hemocytometer method, measurement of sperm concentration by Makler counting chamber is an inaccurate method, especially in semen samples with concentrations less than $40 \times 10^6$.

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REFERENCES

การศึกษาเปรียบเทียบการใช้ Makler counting chamber กับ Improved Neubauer hemocytometer ในภาวะตรวจค่าความเข้มข้นของตัวอสุจิ

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การเปรียบเทียบระหว่างการจึงพันธุ์ในเพศชายส่วนใหญ่จะออกจากการตรวจน้ำอสุจิเป็นหลัก และการตรวจค่าความเข้มข้นของตัวอสุจิก็เป็นการตรวจที่มีความสำคัญมาก การศึกษาโตดีศึกษาความแตกต่างของการตรวจค่าความเข้มข้นของตัวอสุจิโดยใช้ Makler counting chamber โดยตรวจน้ำอสุจิจำนวน 55 ตัวอย่าง ได้แก่ Makler counting chamber และ Improved Neubauer hemocytometer ตามคู่มือขององค์การอนามัยโลก (วิธีมาตรฐาน) จำนวนผลที่ได้ตามวิธีมาตรฐานก่อนทำการตรวจทางสถิติ พบว่าทั้ง 2 วิธีมีความแตกต่างกัน ค่าความเข้มข้นของตัวอสุจิที่ได้จากการใช้ Makler counting chamber ไม่แตกต่างจากค่าที่ได้จากการใช้ Improved Neubauer hemocytometer อย่างมีนัยสำคัญทางสถิติในน้ำอสุจรูปที่มีความเข้มข้นมากกว่า 40x10⁶/มล. แต่การใช้ Makler counting chamber จะได้ค่าสูงกว่า ค่าที่ได้จากการตรวจวิธีมาตรฐานอย่างมีนัยสำคัญทางสถิติ (p < 0.0001) ในน้ำอสุจรูปที่มีความเข้มข้นน้อยกว่า 40x10⁶/มล. จากการศึกษาทั้งหมดพบว่าการใช้ Makler counting chamber จะได้ค่าสูงกว่าวิธีมาตรฐานร้อยละ 11.2 ถึงแม้ว่าการใช้ Makler counting chamber จะมีความสูงกว่าน้อยกว่าการใช้ Improved Neubauer hemocytometer แต่ผลที่ได้จากการใช้ Makler counting chamber ไม่แตกต่าง โดยเฉพาะอย่างยิ่ง เนื่องความเข้มข้นของตัวอสุจิน้อยกว่า 40x10⁶/มล.

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