Original Article

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Comparison of sperm count between the established Makler chamber and the newer *Rohem chamber*

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Abstract

Seminal fluid analysis is used to determine sperm concentration and assess male fertility. This study was designed to compare the sperm-counting efficiency of the Makler and Rohem chambers.

A cross-sectional comparative study was conducted at Teaching Hospital Jaffna from September 2022 to April 2023 among 100 men. Semen samples were analysed using both chambers, and the Bland-Altman plots test was performed to evaluate the agreement between the Rohem chamber and the Makler chamber.

The mean $\pm$ standard deviation (Makler-56.38 $\pm$ 50.01 & Rohem-117.79 $\pm$ 96.04) showed great differences between the both chambers with statistical significance ($p<0.001$). Additionally, the Bland-Altman graph revealed that the majority of the readings were outside the upper and lower limits in the 95% confidence interval, showing a lower chance of clinical agreement between both chambers.

The study findings suggest that using the Rohem chamber instead of the Makler chamber to determine the sperm concentration is suboptimal.

Keywords: Makler chamber, Rohem chamber, Sperm concentration, Seminal fluid analysis.

Introduction

Subfertility is a global health burden affecting roughly one in six adults worldwide (1). Male factors are found to be either solely responsible or to contribute to nearly half of all cases (2). Assessment of sperm concentration and count is an integral part of fertility evaluation and management of these individuals.

The Makler counting chamber with a 10-micron depth is the most widely used sperm counting device, but it is relatively expensive, especially in developing countries. In addition to that, the less expensive Rohem counting chamber with similar depth is also available for sperm counting. Hence this study's objective was to compare the sperm concentrations calculated using these two counting chambers.

Materials and Methods

This was a cross-sectional comparative study conducted with ejaculates from 100 men who were attending the subfertility clinic at the Teaching Hospital Jaffna from September 2022 to April 2023. Azoospermic men were excluded from this study. The participants' ages ranged from 22 to 49 years.

Ethical approval was obtained from the Ethics Review Committee, Faculty of Medicine, University of Jaffna (J/ERC/22/140/NDR/0281). The study was conducted according to the ethical standards of the relevant institutional ethics committee. All study participants provided consent before sample collection in accordance with the institutional review protocol.

The ejaculates were collected through self-masturbation after 4 days of sexual abstinence. Following routine semen analysis, the remaining semen was transferred to a labelled test tube and placed in a beaker containing 3 parts boiling water and 1 part room temperature water for about 20-30 minutes to permanently immobilize all of the sperm. A drop of well-mixed sample was applied to the Makler Chamber, and all sperm within a ten-square area were counted. The counting was repeated on two other different ten-square areas, and the

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numbers were recorded. The three total counts were then averaged. This process was repeated using the Rohem Chamber as well for results comparison.

Statistical analysis

Data were entered in SPSS 21, calculated, and analyzed using descriptive statistics of the mean, standard deviation, range, and 95 percentage confidence level. A comparison of the means of the Makler and Rohem counting chambers using one sample t-test and Bland-Altman plots test was performed to evaluate the agreement between both chambers (3). A paired sample t-test was used to compare the oligozoospermic ejaculates in the Makler chamber with the Rohem chamber.

Results

Table 1 displays the calculated findings about the concentration of sperm. As per the findings, the mean value achieved with the Rohem chamber was significantly ($p < 0.001$) higher than those generated with the Rohem chamber, indicating a substantial difference between the two chambers.

Table (1): Sperm Concentration results (10^6/mL) obtained with Makler and Rohem Sperm Counting Chamber (n=100)

Chamber	Mean ± Standard Deviation	Range	95% confidence	
			Lower	Upper
Makler	56.38 ± 50.01[a]	2 – 284	47.19	67.21
Rohem	117.79 ± 96.04[a]	3 - 567	99.95	138.61
Difference	61.41 ± 57.12	1 - 283	72.74	50.08

[a] Mean value was significantly different from each other ($p < 0.001$)

[b] Difference between individual sperm concentration obtained with Makler and Rohem counting chambers

The samples were further identified as oligozoospermic ejaculates based on the Makler sperm counting chamber of < 15 million/mL (n=18) and compared to corresponding ejaculates based on the Rohem counting chamber (Table 2). Similar to the sperm concentration comparison, the results revealed a large discrepancy between the two chambers, with the mean value obtained with the Rohem chamber being significantly ($p = 0.003$) higher than that obtained with the Makler chamber. Only 7 of the 18 ejaculates were identical between the two sperm-counting chambers.

Table (2): Distribution of ejaculates in Rohem sperm counting chamber based on both chambers

Oligozoospermic ejaculates identified in Makler counting chamber (n=18)

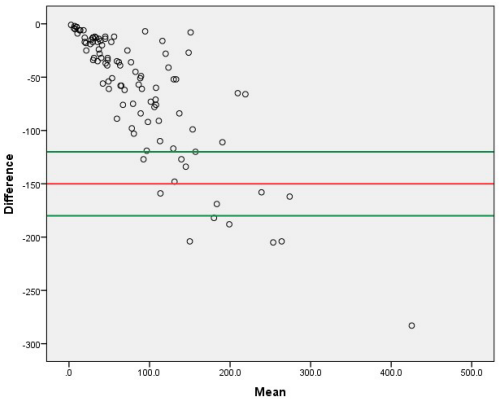
Chamber	Mean ± Standard Deviation	Range	95% confidence	
			Lower	Upper
Makler	9.8 ± 4.1	2 – 15	7.8	11.8
Rohem	28.1 ± 25.6	3 – 104	15.4	40.8

Mean values were significantly different from each other ($p = 0.003$)

7 of the 18 identified as oligozoospermic ejaculates matched.

To evaluate the agreement between the two chambers for clinical assessment in determining the sperm counts, the mean and difference of 100 readings were calculated between the two chambers (Table 1), and a Bland-Altman graph was plotted (Figure 1). One sample T-test performed to find out the difference of the readings showed a mean difference of - 61.41 (SD = 57.117) (Upper = -50.08; Lower = -72.74; CI = 95%). The graph revealed that only a few readings were closer to the mean of the difference, and most were outside the upper and lower limits in the 95% confidence interval. It showed a significantly lower likelihood of using the relatively new low-cost Rohem chamber instead of the existing Makler.

Figure 1: Agreement between sMakler and Rohem chamber in sperm counting method



Discussion

Our objective was to assess the clinical usefulness of the comparatively low-cost Rohem sperm counting chamber with the established Makler chamber, which has been designed specifically for measuring the concentration of human sperm and is very popular, being used by many clinical laboratories following its initial validation against the hemocytometer (4,5). Our findings showed that the sperm concentration determined between the Makler & Rohem chambers was different. The Rohem chamber exaggerated the readings. Also, the clinical agreement between these two chambers was very poor. In a worst-case scenario, one might determine an oligospermic person as normozoospermic, which would result in misdiagnosis. This is not surprising since Zuvela and Matson (2023) (6). reported similar findings with different sperm counting chambers, especially with oligozoospermic samples. This confirms that the sperm concentration determined using any sperm counting chambers will only yield a reliable estimate at best (7).

The current study has strengths along with limitations. It was conducted at the only tertiary hospital in the Northern Province, the representative population of the Northern Province of Sri Lanka. The semen samples were always obtained in the same controlled environment and used both chambers simultaneously. A well-trained andrology research assistant performed all the semen analyses in both chambers. However, we could not recruit more participants due to limited time and resources. Furthermore, to assess the diagnostic efficacy, we compared it with the Makler chamber, as this is commonly employed almost in the entire country. Thus, a larger multicentre diagnostic accuracy study to recruit more participants from the other regions of the country referencing the gold standard method and comparing both Chambers is recommended to have more precise results and diagnostic accuracy.

Conclusion

This study revealed significant discrepancies between the Makler and Rohem chambers, with the Rohem tending to overestimate sperm concentration. As a result, despite its lower price, the Rohem chamber may not be as efficient for sperm counts as the Makler chamber.

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