

## Case Report

# Human Sperm Counting Efficacy of the Shukratara Chamber Versus the Makler Chamber

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## Introduction

Assessment of sperm concentration and sperm count are an integral part of fertility evaluation and management of patients attending a fertility clinic. Although a variety of methods exist for counting sperm, the Neubauer haemocytometer has been widely accepted as the standard for manual counting of human spermatozoa (WHO laboratory manual for the examination and processing of human semen, 2021). Despite being the standard, the haemocytometer is not convenient for analyzing sperm motility and does not work effectively for undiluted samples [1]. To overcome these issues, a 10-micron depth chamber was introduced which enabled the assessment of sperm motility and count simultaneously, the Makler counting chamber [4]. This depth allows for the determination of sperm concentration and motility in undiluted samples [3]. Due to the modification, the Makler counting chamber has proven to be a more convenient method for conducting semen analyses. However, it is relatively expensive, especially for use in developing countries.

The Shukratara sperm counting chamber (HI-TECH Solutions, Malviya Nagar, New Delhi-110017, India) with a 10-micron depth is a less expensive alternative to the Makler counting chamber,

## Abstract

**Purpose:** The objective of this study was to compare the sperm counting efficacy of the less expensive Shukratara sperm counting chamber with the widely accepted Makler counting chamber.

**Methods:** Frozen sperm samples from 70 men were thawed and the sperm concentration determined using both counting chambers respectively.

**Capsule:** The counting efficacy between less expensive Shukratara sperm counting chamber and Makler sperm counting chamber using frozen thawed sperm was found to be similar.

**Results:** The mean  $\pm$  standard deviation ( $67.9 \pm 5.8$  VS  $59.0 \pm 5.9$ ;  $10^6/\text{mL}$ ) and fifth percentile  $\pm$  95 % confidence interval between the two respective chambers for sperm concentration determined. There was no significant difference ( $p > 0.05$ ) between the two sperm concentrations were found.

**Conclusions:** The mean  $\pm$  standard deviation ( $61.9 \pm 72.9$  VS  $62.1 \pm 60.0$ ;  $10^6/\text{mL}$ ) and fifth percentile  $\pm$  95 % confidence interval ( $67.9 \pm 5.8$  VS  $59.0 \pm 5.9$ ;  $10^6/\text{mL}$ ) between the two respective chambers for sperm concentration determined. There was no significant difference ( $p > 0.05$ ) between the two sperm concentrations were found.

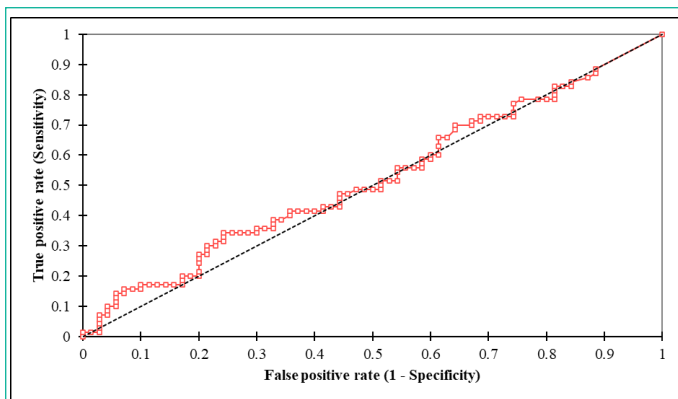
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but untested for counting human sperm. Recently Kumar et al., (2018) and Singh et al., (2022) [2,5] used the Shukratara sperm counting chamber to evaluate bull sperm membrane integrity but failed to determine the efficacy of it in comparison to any other counting chambers. Therefore, the objective of this study was to compare the sperm concentrations and the efficacy of using the Shukratara sperm counting chamber with the Makler counting chamber.

## Materials and Methods

This study was conducted as a laboratory-based cross-sectional study from July to September 2023 at the Andrology laboratory, University unit, Teaching Hospital Jaffna.

Ejaculates obtained by self-masturbation from seventy men who underwent routine semen analysis for fertility workup were recruited to this study after obtaining the patient's informed written consent. As this was a blind study, the health and fertility status of these individuals was not made available to the authors. The leftover ejaculates were kept frozen at  $-18^\circ\text{C}$ .



**Figure 1:** Receiver operating characteristic curve demonstrating the sensitivity and specificity of the Shukratara and Makler Counting Chambers (n=70)\*.

\*The area under the curve is 0.522.

**Table 1:** Comparison of sperm concentration determined with the Shukratara and Makler counting chambers (n=70)\*.

Counting Chamber	Sperm Concentration $\times 10^6/\text{mL}$	
	Mean $\pm$ Standard Deviation	Fifth percentile $\pm$ 95 % confidence interval
Shukratara	61.9 $\pm$ 72.9	67.9 $\pm$ 5.8
Makler	62.1 $\pm$ 60.0	59.0 $\pm$ 5.9

\*student's two-sample t-test revealed no significant difference ( $p < 0.05$ ) between the sperm concentrations obtained for the two sperm counting chambers.

The frozen samples were thawed and the sperm were counted using both chambers per the manufacturer's instructions (Sefi-Medical Instruments). Briefly, a 5 $\mu\text{L}$  volume of thawed and well mixed semen was loaded into each chamber and for consistency, the sperm in the 10 squares of the first, fifth, and tenth rows were counted. The mean and the fifth percentile of the respective counts were also determined based on WHO laboratory manual for the examination and processing of human semen, (2021) guidelines.

### Statistical Analysis

The mean  $\pm$  standard deviation and the fifth percentile  $\pm$  95 % confidence interval were determined for the 70 semen samples, and the two-sample t-test assuming equal variances calculated to compare the difference between the two chambers, with statistical significance determined as being  $p < 0.05$ . A Receiver Operating Characteristic curve (ROC) was performed using XLSTAT 2023.1.6 to assess the clinical accuracy of the Makler and Shukratara chambers, as well as their ability to correctly determine semen concentration. Each chamber was set as a parameter and then evaluated regarding sensitivity and specificity.

### Results

There was no significant difference ( $p > 0.05$ ) in the sperm concentration obtained from the 70 semen samples between the Makler and Shukratara sperm counting chambers (Table 1). The ROC model comparing the two sperm counting chambers is illustrated in Figure 1. The Area Under the Curve (AUC) was 0.522, indicating that there is no difference in the specificity or sensitivity performance when comparing the two chambers.

### Discussion

There was no significant difference in sperm counts from semen using either the Makler or Shukratara counting chambers. Both methods provided similar sperm counts for each of the 70 samples evaluated (Table 1). This lack of difference between the chambers was further confirmed by the ROC curve and the AUC (Figure 1). Based on the AUC, there was no difference in sperm

count between the two chambers when counting dead spermatozoa. Both chambers produced roughly the same result, suggesting that both sperm counting chambers could be viable for use in a clinical setting for analyzing semen samples. Interestingly, significantly no difference found between the mean and the fifth percentile obtained for both counting chambers may suggest for clinical purposes, routinely calculated mean value may be sufficient instead of calculating the fifth percentile as recommended by the WHO laboratory manual for the examination and processing of human semen (2021).

While the hemocytometer method has been recommended as the "gold standard" for assessing sperm concentration by the WHO laboratory manual for the examination and processing of human semen (2021), the Makler chamber has been used in many laboratories worldwide. Despite it being an effective method for counting sperm, the Makler chamber is expensive and therefore may be inaccessible to clinicians and laboratories in developing countries. To overcome the cost of the Makler counting chamber, a Shukratara is a more cost-effective sperm counting chamber was available, however, it has not been used to determine sperm concentration in human ejaculates. In two studies that used the Shukratara, bull sperm was evaluated for different factors such as membrane integrity following cryopreservation for various length of time [2,5]. While both studies used the Shukratara, the accuracy of the counting chamber was not analyzed, making it difficult to apply the Shukratara in a clinical setting.

The present findings demonstrate that when assessing frozen thawed sperm samples, both the Shukratara and Makler did not show significant differences in the sperm concentration. Since the Makler is a widely accepted chamber that may be expensive for developing countries, the Shukratara may prove to be a more financially desirable option that provides similar results. Further studies should explore and compare the Shukratara's sperm counting when analyzing fresh ejaculates to validate the chamber's use in clinical settings.

### Conclusion

There was no significant difference found between the sperm counting efficacy between the Shukratara and Makler counting chambers. Both chambers functioned similarly to one another indicating that the Shukratara sperm counting chamber may be used in lieu of the Makler counting chamber. Despite these results, it is recommended that further studies with fresh ejaculates are used to validate the clinical use of the Shukratara sperm counting chamber.

### Author Statements

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