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Research Article

How Low is too Low? The Makler Chamber's Limit of Detection for Sperm

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Abstract

Commercially available sperm counting chambers do not have an established minimum detectable sperm count, therefore, accurately diagnosing azoospermia has become a daunting challenge. The diagnoses of azoospermia are essential, since individuals are considered sterile if they have no sperm in their ejaculate. Hence, we aimed to clarify and validate the minimum number of sperm per mL which could be detected in the microscopic field of view (MCF) using the Makler counting chamber (Makler Chamber field; MCF). To determine optimal sperm concentrations, fourteen ejaculates were serially diluted with 0.9% (w/v) NaCl to concentrations of 10, 1.0, 0.2, 0.1 and 0.05 x 10^6 sperm per ml. Sperm were only found to be observed within the MCF when the sperm concentration was 0.2 x 10^6 sperm per ml or above. These findings suggest that the minimum detectable sperm concentration for Makler chamber is 0.2 x 10^6 sperm per ml and any sperm concentration below this level could not be detected with confidence.

Keywords: Minimum; Detection; Sperm; Makler Chamber

Introduction

Sperm count is a fundamental factor for male fertility evaluation [1]. Individuals with an absence of sperm in their ejaculate are considered sterile, therefore, assessing the presence of sperm in the ejaculate is essential to the diagnoses of male sterility [2]. Azoospermia affects nearly 1% of the male population and about 10% to 15% of all males with infertility [3,4]. In general, the term azoospermia has been accepted to represent the absence of spermatozoa in the sediment of a centrifuged ejaculate sample [5].

Interestingly, no commercially available sperm counting chambers have a clearly established minimum detectable sperm count. Kiessling et al., [6] offered a theoretically based calculation of the minimum number of sperm to be 1,110 spermatozoa per ml, if one sperm was observed in all nine square grids of the improved Neubauer hemocytometer, and 0.1 x 10^6 spermatozoa per ml if one sperm was found in all 100 square grids of the Makler Chamber. Clinically, this proposed minimum number of sperm would not be acceptable since sperm may be present outside the grid of detection, or more significantly, outside the microscopic field of view.

Our aim is to further clarify and validate the minimum number of sperm/ml which may be detected in the microscopic field of view using a Makler counting chamber.

Materials and Methods

Fourteen ejaculates from apparently healthy men who were referred for semen analysis were obtained by self-masturbation. The ejaculates were then analyzed for semen quality and the remaining left-over samples deidentified, coded and were kept frozen at -18^{0}C until needed. A Nikon Labophot Phase Contrast Microscope with a stage micrometer and 200X magnification was employed to count sperm on the Makler Chamber. The microscopic field diameter was 0.9 mm while the Makler Chamber field (MCF) diameter was 5.5mm.

Frozen samples were thawed, and sperm were counted using the Makler chamber, per manufacturer's instructions (Sefi-Medical Instruments). Briefly, a 5 µL volume of well mixed neat semen was loaded into the chamber and sperm were counted in 10 squares of the first, fifth, and tenth rows of the chamber. If the concentration was found to be low, then sperm in 100 squares was counted and the mean calculated as the concentration per ml. When no sperm were found inside the Makler grid, the numbers were counted in the MCF.

The samples were then diluted with 0.9% (w/v) NaCl to concentrations of 10, 1.0, 0.2, 0.1 and 0.05 x 10^6 sperm per ml, and the sperm concentration was determined as above.

Results

The Mean ± SD of the sperm concentration of the fourteen semen samples was found to be 95.87 ± 33.04 x 10^6 sperm per ml. The results of the various dilutions of the fourteen sperm samples determined using Makler Chamber is detailed in Table 1. The correlation coefficient between the calculated and observed for various sperm concentration was 0.996, with the following additional observations:

- Sperm were present either within the grid or MCF in all fourteen samples when the sperm concentration was 0.2 x 10^6 sperm per ml.
- Sperm were present only in only ten of the fourteen when the sperm concentration was 0.1 x 10^6 sperm per ml.
- Sperm were present only in four of the fourteen samples when the sperm concentration of 0.05 x 10^6 sperm per ml.

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Table 1: Mean ± SD of the diluted Sperm Concentration determined using Makler Chamber.

Mean ± SD Concentration x 10^6/ml*	
Calculated Dilution	**Observed Sperm**
10.0	15.40 ±11.80
1.0	1.411 ±0.72
0.2	0.96 ±0.26
0.1	0.0 ±0.09**
0.05	0.0

*Correlation coefficient between the calculated and observed sperm concentration was 0.996,
**Four of the fourteen samples had no sperm in the MCF.

Discussion

Kiessling et al., [6] calculated the theoretical minimum sperm concentration to be 0.1 x 10^6 sperm per ml if one sperm was found in all 100 square grids of the Makler Chamber. These proposed calculations assumed that there may be sperms outside the grid, if the concentration was found to below this threshold. However, we found sperm within the grid or MCF only when the sperm concentration was a minimum of 0.2 x 10^6 sperm per ml. When the sperm concentration was below this level, four of the fourteen samples had no sperm observed in the MCF, suggesting that the minimum detectable sperm concentration is be 0.2 x 10^6 sperm per ml when using a Makler chamber. Any sperm concentration below this level could not be detected with confidence.

Samples used in this study were frozen, thawed and diluted to achieve the desired concentration. In actual practice, ejaculated semen is a viscous, heterogenous mixture of sperm containing other cells and debris, all of which may affect the distribution of sperm. The correlation coefficient between the calculated and the observed sperm concentration for various dilution was 0.996, suggesting that the dilutions were consistent with reliable observed sperm concentrations (Table 1).

Azoospermia may be suspected if no spermatozoa are observed in replicate wet preparations [7], however Eliasson et al., [5] recommended that an azoospermia diagnosis be made only if no spermatozoa are found in the sediment of a centrifuged sample. Whether or not sperm are found in the sediment depends on the centrifugation time and speed [8,9]. In addition, Corea et al., [10] reported that not all sperm are found in the sediment even when centrifuged at 3000g for 15 minutes.

We found when using the Makler chamber, the minimum detection limit for sperm concentration with a sufficient degree of confidence to be 0.2 x 10^6 or more sperm per ml [11]. Therefore, we conclude that sperm count by Makler chamber alone is not sufficient to label a sample as azoospermic (or an individual sterile) and that additional testing, including the careful assessment of the centrifuged sediment is absolutely mandatory before making such a diagnosis. As additional sperm counting chambers are introduced into the field, similar analyses to ours should be conducted.

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