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"COMMUNICATION"

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EMBRYO-CHAT
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TOPIC OF DISCUSSION

"Semen Analysis Methods"

CHAT DISCUSSIONS COMPILED BY

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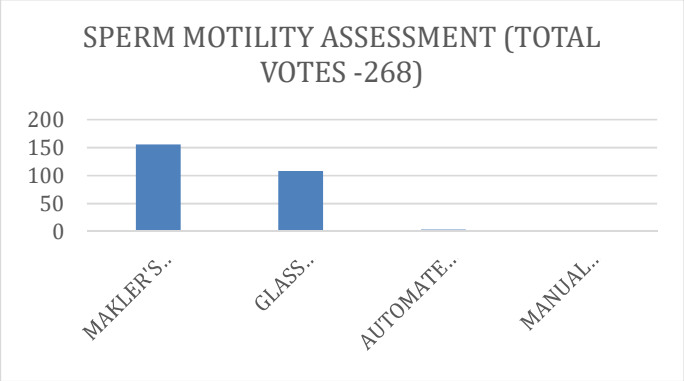
Poll Summary - Semen Analysis Methods (IHERA)

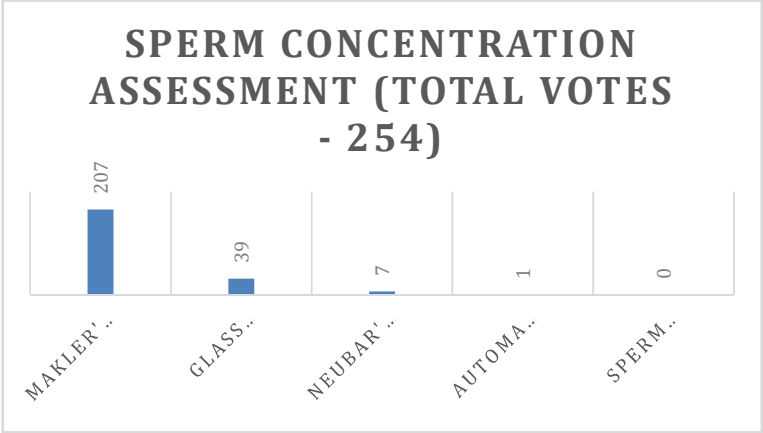
□ Sperm Motility Assessment (Total votes: 268)

- ☑Makler's chamber – 156 votes (58.2%)
- ☑Glass slide method – 108 votes (40.3%)
- □ Automated analyser – 4 votes (1.5%)
- ✖Manual rough calculation & Others – 0 votes



□ Sperm Concentration Assessment (Total votes: 254)

- ☑Makler's chamber – 207 votes (81.5%)
- ☑Glass slide method – 39 votes (15.35%)
- □ Neubauer's chamber – 7 votes (2.76%)
- □ Automated analyser – 1 vote (0.39%)
- ✖Sperm counter – 0 votes



Conclusion:

Makler's chamber remains the preferred method among embryologists for both motility and concentration assessment. The glass slide method is still in use by many, while automated and manual alternatives are far less common.

Semen Analysis Methods: Post Poll Discussion Summary

Introduction

This summary integrates participant perspectives and the supporting literature, particularly focusing on the use of the Makler chamber, Neubauer hemocytometer, glass slide methods, automated analysers, and the importance of standardization in semen analysis practices.

Makler Chamber vs. Glass Slide: Motility and Concentration Assessment

Participants noted that the Makler chamber, engineered for semen analysis, allows for both concentration and motility assessment through a defined grid and precise depth. Its mathematical approach enables quantification of rapid progressive, slow progressive, sluggish, and immotile sperm with a small sample. Many considered it particularly helpful for beginners due to its structure and reduced subjectivity compared to glass slide techniques.

Some felt, however, that handling the Makler chamber—specifically controlling the drop size—can be challenging, especially in settings where micropipettes are not routinely used. Dropsize variation can influence the sperm concentration as there is high possibility to load more sample and participants agreed that if sample volume is not precisely controlled, there is a risk of inaccurate concentration results. It was also highlighted that occasional absence of sperm in the counting grid might be mislabeled as cryptozoospermia, cautioning against hasty interpretation. In busy labs, some argued for glass slide use due to its speed, although at the expense of accuracy.

While a few participants believed the Makler chamber is not ideal for motility assessment, others contradicted this, arguing its grid structure supports more objective motility classification and quantification, consistent with current WHO standards. Studies corroborate that a systematic approach with the Makler chamber provides more accurate and reproducible results than subjective estimates with glass slides.

Glass Slide Method

Some participants still favor the glass slide method for its ease and speed. This method is better preferred to test motility than sperm concentration by our participants and also recommended in the WHO guidelines. According to WHO 6th edition, sperm motility assessment can be done via glass slide method in the following way –

Step 1 – Sample preparation

The sample is liquified within 30 mins of ejaculation at 37 °C. It is mixed gently using a wide bore pipette before making two fresh wet mounts for replicate assessment.

Step 2 – Wet Mount Preparation.

A 10 µl drop of the mixed semen is placed on a clean glass slide and 22X22 mm coverslip is placed on top of the drop in such a way that air bubbles are not formed. It should be ensured that the flow is stopped in one minute, otherwise a new slide should be prepared.

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Step 3 – Microscopy setup

A phase-contrast microscope is used at x200 or x400 magnification for motility evaluation

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Step 4 – Choose fields systematically

Fields within 5 mm of the coverslip edge should be avoided and at least 5 random fields should be selected across the slide.

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Step 5 – Motility Grading and Counting

200 sperms are evaluated per replicated and categorized as

1. **Progressive Motility (PR):**Rapid, linear or large circle motion (>25 µm/s)
2. **Slow Progressive (NP):**Slower, forward motion (5–25 µm/s)
3. **Non-Progressive (NP):** Tail motion without forward head movement (<5 µm/s)
4. **Immotile (IM):**No movement

First PR and NP is counted in each field followed by NP and IM in the same field. This counting is continued until all four categories are sufficiently recorded (≥200 sperm total).

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Step 6 – Report

Each motility category is reported as a percentage and rounded to the nearest whole number. It should be ensured the total across all categories equals 100%.

To analyse sperm concentration, no particular methodology related to glass slide method has been mentioned in WHO 6th edition but still is commonly practiced among clinics. For sperm concentration, a measured volume of semen, not exceeding 10 µl, is placed on a clean glass slide and covered with a standardized 22x22 mm coverslip. Maintaining consistent semen volume and coverslip size ensures a uniform preparation depth—specifically, 20 µm—allowing for accurate analysis. The slide is then examined under a phase contrast microscope at a magnification of 200X - 400 X. A minimum of 200 sperm cells should be counted over multiple fields. It was acknowledged in the discussion and studies also mention that this approach suffers from significant lack of standardization, increased friction that may stress sperm, and substantial inter- and intra-observer variation. Additional challenges include variability and wear in equipment, differences in protocols, and the inherently time-sensitive nature of biological samples.

Makler Chamber vs. Neubauer Chamber

Participants recognized that the Neubauer hemocytometer remains the WHO gold standard for sperm concentration estimation, valued for precision and used primarily for calibration or quality control of the Makler chamber. However, the Neubauer is considered too complex and time-consuming for routine use since it requires semen dilution and technical expertise.

Some participants also emphasized that, in the Neubauer method, use of calibrated pipettes and trained personnel can largely mitigate dilution and counting errors—contributing to its reliability as a reference standard. The importance of using calibrated pipettes and regular laboratory equipment calibration was unanimously highlighted, especially for dilution-based methods like the Neubauer hemocytometer.

A notable operational con of the Makler chamber, as highlighted by participants and confirmed by recent research in a 2025 study in the Asian study of Andrology (Lin Yu et al., 2024), is the critical need for immediate placement of the coverglass after loading the sample. This study notes that even a short delay (5–30 seconds) in applying the coverglass can lead to significant overestimation of sperm concentration (by up to +107%) and progressive motility (by up to +42%) due to central accumulation of motile sperm within the grid area. This issue is unique to the Makler chamber and underscores the importance of strict adherence to correct technique for reliable results

Automated Analysers

Participants emphasized that automated sperm analysers generally align well with Makler's chamber at normal semen concentrations (Baig, Shoebuddin & Ahmed, 2019), but become increasingly unreliable at higher concentrations due to factors like cellular debris as automated analysers measure sperm conc based on optical density and cellular debris can alter the density measured v/s optical density (Mortimer & van der Horst, 2015). Also they are more costly and all centres cannot afford it. Participants agreed that they work best only for normal parameters and need to be fed more data to become more accurate

Clinical and Classification Practices

Participants agreed that the WHO 2021 motility classification provides more actionable information than earlier methods, particularly for preparation and selection in IUI and IVF.

Since the non-progressive sperm are less likely to reach the egg after processing, for IUI, there was a discussion on whether the decision be based on total motility or total progressive motility (TPM) specifially. The recommendation was made to calculate Total Progressive Motile Sperms as:

TPMS = sperm concentration × semen volume × progressive motility (%) rather than TMSC. This could be a more clinically relevant measure for IUI planning.

For ICSI, while motility plays a role, morphology is considered even more critical. When facing samples with completely immotile sperm, participants recommended performing the Hypo- Osmotic Swelling (HOS) test to select viable sperm for ICSI—a practice confirmed as effective by clinical protocols.

Standardization

There was consensus on the need for routine cross-validation: comparing 30–50 samples in parallel using both Makler and Neubauer chambers, calculating 95% confidence intervals, and establishing correction factors specific to each laboratory setup.

Conclusion

Participants largely favor the Makler chamber for routine analysis due to its scientific foundation, reliability, and objectivity, while remaining attentive to its operational limitations—especially the need for immediate coverglass placement to avoid major overestimation errors. Regular quality control against the Neubauer standard and careful technique are essential for optimal clinical and scientific practice in semen analysis.

References

1. Baig, A. S., Shoebuddin, . M. & Ahmed, . M. (2019) Comparison of manual sperm analysis with computer-assisted sperm analysis: A comparative cross-sectional study. Natl J Physiol Pharm Pharmacol, 9 (9), 862-864. doi:10.5455/njppp.2019.9.0621817062019

2. Yu L, Cheng QY, Jia YL, Zheng Y, Yang TT, Wu YB, Li FP. Delayed covering causes the accumulation of motile sperm, leading to overestimation of sperm concentration and motility with a Makler counting chamber. Asian J Androl. 2025 Jan 1;27(1):59-64. doi: 10.4103/aja202474. Epub 2024 Sep 3. PMID: 39225004; PMCID: PMC11784962.

3. Mortimer, S. T., van der Horst, G., & Mortimer, D. (2015). The future of computer-aided sperm analysis. Asian journal of andrology, 17(4), 545–553. https://doi.org/10.4103/1008- 682X.154312

4. World Health Organization. (2021). WHO Laboratory Manual for the Examination and Processing of Human Semen (6th ed.). Geneva: World Health Organization

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