A prospective study of variations in conventional semen parameters among local andrology laboratories

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Abstract

Introduction: Wide variations in semen parameters exist among different andrology laboratories. This study sought to determine the inter- and intra-technician variations in conventional semen parameters among local andrology laboratories.

Methods: Pooled semen samples were prepared and sent in two batches to participating andrology laboratories. One technician who routinely performed semen analysis in the participating laboratories was asked to analyze the study samples. The inter-technician and intra-technician coefficients of variation (CVs) were calculated. Information on the qualification and training of the participating technicians, the workload of the centers, their techniques and participation in external quality assurance programs were collected and correlated with the CVs.

Results: Eleven andrology laboratories participated in the study. The inter-technician CVs ranged from 14.3% to 44.1% for concentration, 13.8% to 26.2% for progressive motility, and 38.8% to 95.3% for morphology. Andrology laboratories which participated in external quality assurance programs had lower inter-technician CVs for concentration ($P = 0.004$) and progressive motility ($P = 0.002$), but not for morphology ($P = 0.232$). Technicians with more experience or higher workload did not demonstrate lower intra-technician CVs.

Conclusion: There were considerable inter- and intra-technician variations in the assessment of sperm concentration, progressive motility and morphology among local andrology laboratories, independent of the workload and experience of the technicians. Participation in external quality assurance programs reduced inter-technician variations in sperm concentration and progressive motility but not morphology.

Keywords

Semen analysis; Inter-technician variation; Intra-technician variation

1. Introduction

Infertility is a common problem affecting 1 in 6 heterosexual couples [1]. Males are the main cause of failure to conceive in 20 to 30% of couples with infertility [2, 3].

Semen analysis is an important factor in the management of infertile couples. It provides information on the etiology and various treatment options for couples suffering from infertility. There is a wide variation in semen parameters amongst samples from the same individual [4]. Possible explanations are inherent fluctuations of semen qualities, and inter- or intra-technician variability [3, 4]. In order to minimize the variations in performing semen analysis, the
World Health Organization (WHO) recommends that each andrology laboratory implement a quality assurance program to monitor sperm concentration, motility and morphology [5–8].

In Hong Kong, it is common for infertile couples to consult multiple doctors and undergo semen analysis on multiple occasions in different andrology laboratories. This study sought to determine the inter- and intra-technician variations in conventional semen parameters among local andrology laboratories.

2. Methods

Fifteen andrology laboratories affiliated with assisted reproduction centers were invited to participate in the study and the director of each participating laboratory signed the consent form. A questionnaire, an instruction manual, and a standardized report form were sent to each of the participating laboratories. The questionnaire collected information on the workload for semen analysis, qualification and training of technicians performing semen analysis, the techniques adopted, as well as the internal or external quality assurance programs used in the participating laboratories.

The Andrology Laboratory of the Centre of Assisted Reproduction and Embryology, and the University of Hong Kong-Queen Mary Hospital (HKU-QMH CARE) prepared the study semen specimens in two batches, which were sent to the participating laboratories. In each batch, five pooled semen samples were prepared from residual samples donated by men after routine semen analysis as part of investigations for infertility at HKU-QMH CARE. The semen samples were produced by masturbation after an abstinence period of 2 to 7 days. Written consent to use the residual semen samples for research use had been obtained. These pooled samples were aliquoted and sent to the participating laboratories for determination of sperm concentration, motility and morphology. All the specimens were labeled with a unique identification code. The laboratories were blinded to the origin of the samples. The same exercise was repeated on another batch of five samples after three months.

The specimens with normal parameters were diluted in a preservation solution to determine sperm concentration (human serum albumin [Irvine Scientific, Santa Ana, CA], sodium chloride [Sigma Chemical, St Louis, Mo], Triton X-100 [Sigma], polyvinyl-pyrrolidone [Sigma], silicone antifoam [Sigma] and sodium azide [Sigma]) before being sent to the participating laboratories [9]. Counting was performed according to the usual protocol of each of the laboratories. For motility assessment, the percentage of progressively motile, non-progressively motile and immotile sperm were analyzed on video clips prepared from the specimens; each video clip consisted of five to ten frames of randomly selected fields of a wet preparation, with each frame lasting for 15 seconds. For preparation of the video clips, the semen samples were diluted with culture medium (Eagles Balanced Salt Solution [Sigma]) supplemented with 10% (v/v) human serum albumin (Irvine Scientific) so that there were approximately 40-60 sperm per high power field. The total length of each video clip did not exceed two minutes. Five video clips, one from each pooled specimen, were copied onto a DVD and distributed to the participating centers for analysis. For sperm morphology assessment, air-dried and unstained sperm smears on cleaned and labelled glass slides were prepared using the feathering technique [5]. The participating laboratories were required to stain the slides using their own protocol. For laboratories that did not usually use staining solutions for morphological assessment, digital images of stained sperms were provided. The images were captured from semen samples that were air-dried, methanol-fixed and stained by the Diff Quik staining solution [10]. The same semen samples that were used to prepare the unstained slides were used to prepare the digital images. All participating laboratories were requested to perform morphological analysis on the images. Since all the laboratories did not routinely perform staining in their practice, CVs for morphology assessment were analysed based on the results from the digital images.

All samples were transported at room temperature and arrived at the participating laboratories on the same day. The participating laboratories were advised to keep the samples refrigerated (4-8 °C) on arrival. One technician who routinely performed semen analysis in the participating laboratories was asked to use the usual methods and criteria of the participating laboratories to analyze the study samples. All results were reported and sent back to the principal investigator in a standardized format.

Ethics approval was obtained from both the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. The study was registered in the HKU Clinical Trials Registry with the trial number HKUCTR-2057.

3. Statistical analysis

The coefficient of variation (CV) was calculated as 100% x standard deviation/mean. Wilcoxon Signed-rank test was used for comparison of inter-technician CVs between groups. Mann Whitney U test was used for comparison of intra-technician CVs between groups. Continuous variables are expressed as median (25th-75th percentile). A two-tailed value of P < 0.05 was considered statistically significant. Statistical analysis was performed using the IBM SPSS version 25.0 (IBM Corp, Armonk, NY, United States).

4. Results

Eleven andrology laboratories participated in the study and completed two batches of semen analysis (Table 1). There were large variations among the technicians regarding workload (2 to 40 semen samples analyzed per week) and years of experience (1 to 28 years). Three technicians had Medical Laboratory Technologist (List 1) (MLT-I) registration and eight technicians did not. MLT-1 is an official registration for medical laboratory technicians in Hong Kong. Eight laboratories provided in-house training, one laboratory provided both in-house and overseas training, one laboratory pro-
TABLE 1. Characteristics of the 11 participating laboratories and their semen analysis procedures.

<table>
<thead>
<tr>
<th>Criteria or standard for semen analysis using WHO Criteria 2010 (5th edition manual)</th>
<th>Number of laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semen volume assessment (graduate pipette)</td>
<td>11</td>
</tr>
<tr>
<td>Sperm concentration measurement</td>
<td>11</td>
</tr>
<tr>
<td>Improved Neubauer Cytometer</td>
<td>7</td>
</tr>
<tr>
<td>Makler Cytometer</td>
<td>4</td>
</tr>
<tr>
<td>Sperm motility assessment (Manual counting)</td>
<td>11</td>
</tr>
<tr>
<td>Staining method for morphology assessment</td>
<td></td>
</tr>
<tr>
<td>Diff-Quik</td>
<td>6</td>
</tr>
<tr>
<td>Testimplets Pre-stain slide</td>
<td>5</td>
</tr>
<tr>
<td>Average number of semen analysis per week</td>
<td></td>
</tr>
<tr>
<td>&lt; 10</td>
<td>2</td>
</tr>
<tr>
<td>10-20</td>
<td>3</td>
</tr>
<tr>
<td>21-40</td>
<td>6</td>
</tr>
<tr>
<td>Number of staffs involved in semen analysis</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>4</td>
</tr>
<tr>
<td>3-5</td>
<td>5</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>2</td>
</tr>
<tr>
<td>Participation in external quality assurance program</td>
<td></td>
</tr>
<tr>
<td>CAP</td>
<td>3</td>
</tr>
<tr>
<td>UK NEQAS</td>
<td>2</td>
</tr>
<tr>
<td>FertAid-QAP</td>
<td>1</td>
</tr>
<tr>
<td>Gamete Expert</td>
<td>1</td>
</tr>
<tr>
<td>Do not participate in any external quality assurance program</td>
<td>4</td>
</tr>
</tbody>
</table>

CV: Collage of American Pathologist.
UK NEQAS: United Kingdom National External Quality Assessment Service.
FertAid-QAP: FertAid-Quality Assurance Program.

provided both in-house training and training from another local university unit, and one laboratory provided only overseas training. All laboratories used manual counting and scoring for concentration, motility and morphology assessment. For motility assessment, seven laboratories used wet preparation and the other four used the Makler counting chamber. For morphological assessment, all participating laboratories followed the WHO Manual (2010) [5].

4.1 Inter-technician variations for individual samples

Table 2 shows the median (25-75th percentile) and inter-technician CVs for concentration (million/ml), progressive motility (%), and morphology (%) of each sample. Samples A to E were distributed in the first batch, whereas samples F to J were distributed in the second batch. The inter-technician CVs ranged from 14.3% to 44.1% for concentration, 13.8% to 26.2% for progressive motility and 38.8% to 95.3% for morphology. The mean inter-technician CVs for concentration, progressive motility and morphology were 25.85%, 18.54%, and 35.38% respectively. Semen analysis data for concentration, progressive motility and morphology of the ten samples reported by the 11 technicians are shown in Supplementary Table 1,2,3.

For assessment of sperm concentration, seven laboratories used the Neubauer chamber as recommended in the WHO Manual, while four laboratories used the Makler chamber. There was no significant difference in the inter-technician CVs for sperm concentration between laboratories using the Neubauer chamber and those using the Makler chamber (P = 0.114). Laboratories with participation in external quality assurance programs had lower inter-technician CVs for concentration (P = 0.004) and progressive motility (P = 0.002), but not for morphology (P = 0.232), compared to laboratories without these programs (Table 3).

4.2 Intra-technician variations

Samples F, G and J were duplicate samples for determining the intra-technician variations, and were blinded to the participating technicians. The intra-technician CVs ranged from 5.23 to 53.18% for concentration, 2.49% to 15.32% for progressive motility and 16.10% to 66.62% for morphology. The mean intra-technician CVs were 18.54%, 7.31% and 35.38% for concentration, progressive motility and morphology respectively (Fig. 1). Technicians with three or less years of experience and those with more than three years of experience did not differ significantly in intra-technician CV for progressive motility (P = 1.00). The CVs in technicians with more than three years of experience were significantly higher than those with three or less years of experience for both concentration (P = 0.048) and morphology (P = 0.024). There was no significant difference in intra-technician CVs for concentration (P = 0.279), progressive motility (P = 0.776) or morphology (P = 0.279) in technicians who performed more than 20 tests per week compared to those who had 20 or less tests per week.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (x 106/mL)</th>
<th>CV (%)</th>
<th>Progressive motility (%)</th>
<th>CV (%)</th>
<th>Morphology (%)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10.7 (7.1-12.0)</td>
<td>27.2</td>
<td>37.0 (35.0-39.0)</td>
<td>19.7</td>
<td>11.0 (7.0-16.0)</td>
<td>41.5</td>
</tr>
<tr>
<td>B</td>
<td>48.0 (43.0-56.0)</td>
<td>20.4</td>
<td>24.0 (22.0-28.0)</td>
<td>23.2</td>
<td>2.0 (1.0-4.2)</td>
<td>64.6</td>
</tr>
<tr>
<td>C</td>
<td>29.4 (25.5-34.0)</td>
<td>25.2</td>
<td>42.0 (37.9-44.0)</td>
<td>22.2</td>
<td>5.0 (3.0-8.0)</td>
<td>45.0</td>
</tr>
<tr>
<td>D</td>
<td>39.1 (27.5-42.0)</td>
<td>24.4</td>
<td>45.0 (42.0-53.0)</td>
<td>20.5</td>
<td>6.0 (4.0-9.0)</td>
<td>43.0</td>
</tr>
<tr>
<td>E</td>
<td>37.0 (25.0-45.0)</td>
<td>28.9</td>
<td>50.3 (48.0-55.0)</td>
<td>13.8</td>
<td>4.0 (2.0-5.0)</td>
<td>44.1</td>
</tr>
<tr>
<td>F</td>
<td>41.6 (37.0-59.0)</td>
<td>31.7</td>
<td>71.0 (54.0-74.0)</td>
<td>21.5</td>
<td>6.0 (3.0-9.0)</td>
<td>66.5</td>
</tr>
<tr>
<td>G</td>
<td>44.3 (39.0-47.0)</td>
<td>17.3</td>
<td>61.0 (53.0-68.0)</td>
<td>16.7</td>
<td>6.0 (4.0-7.0)</td>
<td>64.1</td>
</tr>
<tr>
<td>H</td>
<td>18.0 (16.3-20.5)</td>
<td>14.3</td>
<td>38.0 (37.0-43.0)</td>
<td>16.7</td>
<td>6.0 (4.0-6.9)</td>
<td>38.8</td>
</tr>
<tr>
<td>I</td>
<td>134.0 (99.0-163.0)</td>
<td>25.0</td>
<td>15.0 (11.4-18.0)</td>
<td>26.2</td>
<td>1.0 (0.0-2.0)</td>
<td>95.3</td>
</tr>
<tr>
<td>J</td>
<td>51.1 (40.3-60.5)</td>
<td>44.1</td>
<td>67.0 (55.0-69.0)</td>
<td>1.3</td>
<td>3.7 (2.0-6.0)</td>
<td>57.8</td>
</tr>
</tbody>
</table>

There was no significant difference in the CV from samples in the first batch of semen patients compared to samples in the second batch ($P > 0.05$).

5. Discussion

This prospective study involving conventional semen parameters amongst andrology laboratories found that the inter-technician CVs were 14.3% to 44.1% for concentration, 13.8% to 26.2% for progressive motility and 38.8% to 95.3% for morphology. A previous study reported that among 10 laboratories, the inter-laboratory CVs were 23% to 73% for concentration, 21% for motility, and 25% to 87% for morphology [11]. Another study reported inter-laboratory CVs among 20 laboratories was 29% to 52% for concentration, 39% to 71% for motility and 17% to 26% for morphology [12]. Our reported CVs for concentration and motility appeared to be lower than the previous studies, and morphology appeared higher than previously reported. Morphology assessment is highly technician-dependent even though a unified protocol, the WHO manual, was used by all the laboratories. Clinicians need to take into account such large variations when interpreting semen analysis reports and managing infertile couples.

A study from Italy reported that the lowest bias values for morphology and concentration were in laboratories with a workload of more than 200 semen analyses per year [13]. In our study, the CVs for sperm concentration, progressive motility and morphology were not reduced by more experienced technicians or those who performed more than 20 semen analyses per week. This may due to the small sample size which did not confer adequate statistical power to detect any differences. Nonetheless, years of experience or volume of workload was not directly related to CVs. The exact methodological protocol and quality control of the laboratory may be a more important factor in determining the accuracy of semen analyses. It suggested that external quality assurance programs may help to reduce inter-technician CVs [14]. Our data demonstrated significantly lower inter-technician CVs for concentration and progressive motility in centers participating in external quality assurance programs. There was a trend for lower inter-technician CVs for morphology, though statistical significance was not achieved, probably due to the small sample size, and that variations in assessment of morphology are generally greater than that of concentration and motility.

This is the first study on the variations of conventional semen analysis results among local andrology laboratories. Possible bias and errors can be reduced if all technicians perform semen analysis on fresh samples in the same location at the same time, and allowing the technicians to do the analyses in their own laboratories.

Since semen analysis plays a key role in determining both the diagnosis and treatment options for infertile couples, clinicians need to be aware of the variability when interpreting the results from semen analyses. In cases of abnormal results, a repeat analysis is recommended according to the NICE guideline. Training workshops may help to standardize the performance and minimize the variations among different technicians working in different laboratories [15]. A previous study in Denmark has shown that quality control workshops could improve standardization of sperm concentration and motility assessment between centers [15].

6. Conclusions

There were considerable inter- and intra-technician variations in the assessment of sperm concentration, progressive...
motility and morphology among local andrology laboratories, independent of the workload and experience of the technicians. Participation in external quality assurance programs reduced inter-technician variations in sperm concentration and progressive motility but not morphology.

**Author contributions**

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity. Concept or design: MT Lam, KKW Lam, EHY Ng, WSB Yeung, RHW
Li Acquisition of data: KKW Lam, CHY Lock
Analysis or interpretation of data: MT Lam, KKW Lam, RHW Li
Drafting of the manuscript: MT Lam
Critical revision for important intellectual content: all authors.

Ethics approval and consent to participate
The study protocol was reviewed and approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. (Ref no. UW 16-120)

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- The University of Hong Kong - Family Planning Association Andrology Laboratory
- Hong Kong Assisted Reproduction Centre
- Hong Kong Reproductive Medicine Centre
- ProlIVFic A.R.T. Centre
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Conflict of interest
All authors have disclosed no conflicts of interest.

Implications for clinical practice or policy
Clinicians should take the variation of conventional semen parameters into account when interpreting semen analysis reports from different andrology laboratories. Participation in external quality control assurance programs reduced inter-technician variability.

Supplementary material
Supplementary material associated with this article can be found, in the online version, at https://oss.jomh.org/jomh/article/1356881320863514624/attachment/Supplementary%20Tables.docx.

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