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## A continuous quality control program for strict sperm morphology [Male Factor]

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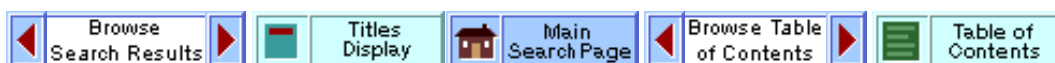
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### Abstract

Objective: To develop a training program with intervals of continuous quality control assessments for the evaluation of strict sperm morphology.

Design: Prospective analytical study.

Setting: Academic hospital and academic institution setting.

Patient(s): Healthy sperm donors.

Intervention(s): Nine individual andrology laboratories in Switzerland were invited to participate in a training course for strict sperm morphology, which was followed up every 3 months by a continuous quality control program. Each laboratory received six slides over a period of 9 months, during which time the results were forwarded to the reference laboratory. Papanicolaou stain sperm slides were prepared and shipped to participating laboratories every 3 months.

Main Outcome Measure(s): Percentage of normal spermatozoa.

Result(s): The mean ( $\pm$ SE) percentage of normal sperm reported by the reference laboratory compared with the participating laboratories for slides 1-6 were  $11.4 \pm 1.6$  vs.  $17.3 \pm 6$  ( $P > .2$ ),  $6.0 \pm 1.3$  vs.  $8.6 \pm 2.5$  ( $P > .2$ ),  $9.0 \pm 0$  vs.  $9.6 \pm 3$  ( $P > .2$ ),  $1 \pm 0$  vs.  $1.2 \pm 0.2$  ( $P > .2$ ),  $23.3 \pm 0.3$  vs.  $28.0 \pm 1.3$  ( $P > .2$ ), and  $2.0 \pm 0$  vs.  $6.1 \pm 1.2$  ( $P > .2$ ), respectively. Technician proficiency was reported to differ by  $<10\%$  from the reference laboratory in 94% of cases.

Conclusion(s): The results illustrate that training and proficiency testing can be conducted on a national and international level with the support of a reference laboratory. Global quality control measurements in andrology laboratories should become mandatory, since these results indicate that continuous quality control for laboratory technicians can be successful.

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Sperm morphology is possibly the sperm variable most consistently related to in vitro fertilization success (1-4). A logistic regression model, including DNA status and sperm morphology, revealed that sperm morphology (strict criteria) and the concentration of progressive motile sperm were the principal predictors for in vitro fertilization. (5-9). The value of sperm morphology assessments as a predictor of a man's fertilizing potential has often been challenged because of different classification systems. Several factors are responsible for technical variation, including differences in the methods used to prepare and stain specimens (10, 11), differences in proficiency among technicians (12-15), and inherent differences in classification criteria and methods (1, 16, 17).

Previous studies have reported significant variation in the percentage of normal sperm when different observers and laboratories (18, 13-15, 19) analyze specimens. Thus, comparison of the predictive power of semen parameters from one setting with that of another setting was always problematic, as was the attempt to transfer the specific values to fit the patient population.

Based on the growing need for quality control measurements in andrology and especially for sperm morphology, the Tygerberg Reproductive Biology Research Laboratory developed a strict-criteria sperm morphology training program for technicians, aiming to record and evaluate the efficiency of continuous sperm morphology training. The present report aims to assess the value of continuous quality control on technician accuracy.

## MATERIALS AND METHODS

### Training

Institutional review board approval was obtained before the onset of the study. Participants received intensive training in strict sperm morphology evaluation during a 1-day sperm morphology workshop presented by T. F. Kruger. All participants were provided with a take-home reference set of five prestained Papanicolaou slides, which included slide 1, which contained sperm from a donor with > 14% normal forms (this slide was also used during pretraining evaluation), and slide 2, which contained sperm with <4% normal forms.

Slides 1 and 2 were repeatedly used until each participant was familiar with the shape and microscopic appearance of the so-called normal sperm according to strict criteria. Slides 3, 4, and 5, used during the teaching course, contained sperm samples with >14%, 5%-14%, and ≤4% normal forms, respectively. The latter slides served as controls to evaluate the participant's knowledge of normal sperm cells. For a spermatozoon to be considered normal, the sperm head, neck midpiece, and the tail must be normal. The head should be oval in shape.

Allowing for the slight shrinkage that fixation and staining induce; the length of the head should be 4.0-5.0 μm, and the width 2.5-3.5 μm; the length-to-width ratio should be 1.50 μm to 1.75 μm. These ranges are the 95% confidence intervals limits for Papanicolaou-stained sperm heads (11). Estimation of the length and width of the spermatozoa were made with an ocular micrometer. There should be a well-defined acrosomal region making up 40%-70% of the head area. The midpiece should be slender, less than 1 μm; 5× the length of the head, and it should be attached axially to the head. The tail should be straight, uniform, thinner than the midpiece, uncoiled, and approximately 45 μm long (20).

This classification scheme requires that all "borderline" forms be considered abnormal (21, 15). Papanicolaou staining was employed in the study since the authors use the stain as the standard staining method (15).

### Continuous Quality Control (CQC) System

Participants were requested to enroll in the CQC system, which entailed the following:

1. A communication line via a FAX/E-mail facility, through which each enrolled laboratory obtained direct access to advice and slide information to ensure the continuous educational value of the CQC system.
2. On a quarterly basis, each enrolled laboratory received a set of two Papanicolaou prestained slides. Each slide set contained sperm obtained from normo-, terato-, and/or severe teratozoospermic sperm samples. Each slide was evaluated for percentage of normal cells by the reference laboratory prior to shipment.

Participants had to record the percentage of normal cells for this slide and forward the results to Tygerberg Hospital, where all the information is recorded in a database. The "correct" results according to the reference laboratory, i.e., the percentage of normal forms present on each of the slides, were subsequently supplied to the participating laboratory. Each participating laboratory received six slides over a period of 9 months. The first set of slides was shipped 3 months after the training course; a total of 54 slides were sent to the nine participating laboratories.

### Evaluation of Quality Control Program

The results of the participants' morphology evaluation at each stage of the evaluation program were compared with the evaluation of the reference laboratory, and the difference in scores was calculated. Results were expressed by comparing the mean percentage of normal forms on each of the reference slides shipped to the nine laboratories with the mean reported by the participating laboratories.

Using the reference laboratory results as standards, technician proficiency was expressed as the mean percentage difference between the participating and reference laboratory, calculated from the separate results of six slides (three sets of two slides) recorded over a 12-month post-training period.

## Statistical Analysis [↑](#)

The paired Student's *t*-test was used to calculate the statistical significance of the differences recorded between the reference and participating laboratories. The technician proficiency was reported in a scattergram by use of the mean percentage difference between the participating and reference laboratory for each slide.

## RESULTS [↑](#)

Comparison between the mean percentage of normal forms recorded by the reference laboratory compared with that of the participating laboratories for each of the three sets of two slides (i.e., six slides) is depicted in [Figure 1](#). The mean ( $\pm$ SE) percentage of normal sperm reported by the reference laboratory compared with participating laboratories for slides 1-6 were  $11.4 \pm 1.6$  vs.  $17.3 \pm 6$  ( $P > .2$ ),  $6.0 \pm 1.3$  vs.  $8.6 \pm 2.5$  ( $P > .2$ ),  $9.0 \pm 0$  vs.  $9.6 \pm 3$  ( $P > .2$ ),  $1 \pm 0$  vs.  $1.2 \pm 0.2$  ( $P > .2$ ),  $23.3 \pm 0.3$  vs.  $28.0 \pm 1.3$  ( $P > .2$ ), and  $2.0 \pm 0$  vs.  $6.1 \pm 1.2$  ( $P > .2$ ), respectively.

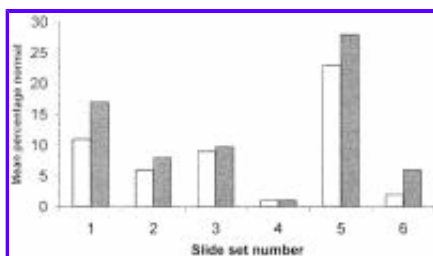


FIGURE 1 Comparison between the mean percentage of normal forms recorded by the reference laboratory (**open columns**) and that of the participating laboratories (**shaded columns**).

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The mean percentage of normal forms that were reported by the participating laboratories for each of the six slides did not differ significantly from the results that were recorded by the reference laboratory. Technician proficiency was calculated by the mean percentage difference between the reference and participating laboratory for the six slides and the nine participating laboratories ([Fig. 2](#)). The mean percentage difference reported for 51 (94%) slides was  $<10\%$  from the mean percentage of normal forms reported by the reference laboratory.

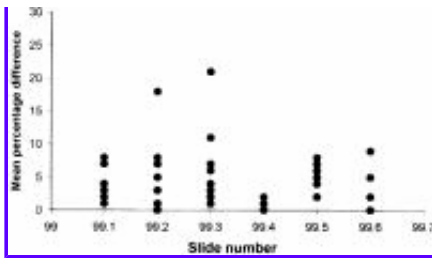


FIGURE 2 The mean percentage difference recorded for six slides between the reference and the nine participating laboratories.

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## DISCUSSION [↑](#)

Despite the efforts to standardize, sperm morphology remains one of the most controversial semen parameters in terms of its role in male fertility potential (1, 7, 8). Evaluation of the percentage of normal sperm morphology features with light microscopy is subjective and therefore difficult to compare between laboratories or even within laboratories, since different techniques of assessing sperm morphology have been extensively used and published (17, 20-23).

Cooper et al. (23) described the results from their external quality control program for semen analysis during which formalin-fixed semen samples and videotapes of motile sperm were distributed every 3 months to participating laboratories over a period of 3-4 years. The initial tendency of the participants in that study to report lower values for the percentage of normal forms was later abolished and as a result closer agreement was reached. The most prominent problem in morphology classification and morphology scoring is the large variation coefficient that exists between and among different technicians in different laboratories.

Despite the problems associated with the preparation of slides and staining methods, the use of different classification systems, and the subjective nature of visual sperm morphology assessment, we still believe in the power of this important parameter in routine semen analysis. This is true both for first world laboratories that need constant quality control measurements and especially for laboratories in developing countries where sophisticated diagnostic laboratories are not readily available.

Individual scatterplots of the results from the six slides used for each participant (Fig. 2) demonstrate the power of CQC measurements. The results for slide 99.1 from one laboratory was omitted from Figure 2 since it was clearly an outlier, differing by 60% from the reference laboratory's result for that slide. The specific individual clearly benefited from the CQC program since the scores reported improved constantly, until the difference was <10%.

The scatterplots may also be very useful in providing a specific laboratory with information regarding technician quality over extended periods. Training projects on international and national levels can provide a useful method to record sperm morphology evaluation standards. International reference andrology laboratories will be able to monitor the technical quality and performance of each participating laboratory. Changes in individual standards can immediately be identified, and corrective measurements can be taken to address the problem.

The results of the present study are encouraging since the nine participants, once trained, maintained for a period of at least 9 months a high level of accuracy in their morphology assessments. The difference between reference laboratory scores compared with the participating laboratory values were in most cases < 10% (Fig. 1). Training of technicians and CQC will ensure maintenance of repeatable sperm morphology data. Participating laboratory number 3 provides an example of the importance of reference laboratory feedback.

This will inevitably lead to an improvement in the diagnosis of male-factor infertility, which will lead to a refinement in the therapeutic approach. Moreover, proficiency testing of technician skills is of the utmost importance if andrology laboratories want to operate under a professional code of conduct. Morphology evaluation by strict criteria requires intensive training. In our experience, continuation of controlled evaluation over extended periods by a reference laboratory provides satisfactory results as far as technician quality is concerned. Sperm morphology training programs are of the utmost importance in all andrology laboratories, especially since the literature clearly indicates that improper technical skills can deprive clinicians of the ability to make a proper diagnosis.

The authors firmly believe that global quality control measurements in andrology laboratories are mandatory in the next millennium. A high-quality semen analysis is still the cornerstone of an investigation of an infertile couple. In order to maintain low intratechnician and intertechnician variation and high-quality proficiency testing among laboratory technicians, continuous teaching programs should be available to all.

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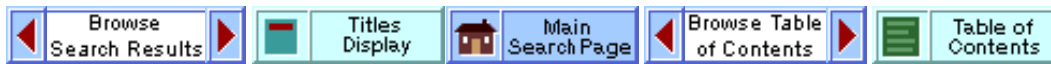
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