**INTRODUCTION AND AIMS**

The assessment of sperm morphology is subjective and different classification systems have been used around the world over time. In an attempt to standardize all methods used in the analysis of semen, the World Health Organization (WHO) produced its first manual in 1980, revising it in 1987, 1992 and 1999, culminating in the current version 5th Edition (WHO5) produced in 2010. Interestingly, a draft of the 6th Edition has just been sent out for public review.

The classification of normal sperm has varied from the early liberal methods whereby MacLeod and Gold (1951) were “not prepared at this time to classify any but the most distorted forms as truly abnormal” to Gold, Andriole, and Gold (1969) who “not prepared at this time to classify any but the most distorted forms as truly abnormal”. This contrasts with the WHO5 that states that we should “limit what is identified as normal to the potentially fertilizing subpopulation of spermatozoa prevalent in endocervical mucus”. The method recommended in WHO5 is a simple normal/abnormal classification, and all borderline forms are to be considered abnormal.

The aims of the present study were to (i) identify changes in morphological criteria used by clinical laboratories since before the release of WHO5, (ii) determine the consistency with which laboratories identify sperm with normal morphology since the release of WHO5, and (iii) assess the between-laboratory precision over time.

**MATERIALS AND METHODS**

Samples were posted to laboratories enrolled in the sperm morphology module. Distributions were made every three months according to a pre-determined schedule, and each distribution included 3 ‘wet’ semen samples and a slide with a semen smear stained with Diff Quik. Results were submitted online by laboratories via the EQASRM website within five weeks of sample dispatch, and statistical summaries of the results were then available for download after the closing date. In addition to the proportion of sperm with normal morphology, laboratories were asked to state the lower reference limit used when reporting clinical samples, and these then linked to the de-identified morphology classification system used. Summaries of the results for the pre-stained slides were excluded from this study to ensure the laboratories were analysing samples stained under their routine protocols.

Labs, including those in the study, used a lower reference limit of 4% normal forms, and this is described in the WHO 5th Edition manual. That classification of normal sperm morphology is said to align closely with the Strict criteria.

Labs were asked to report for each sample the proportion of sperm with normal morphology. The sample summaries supplied back to the laboratories contained the following information: (i) the all laboratory-trimmed mean (ALTM) and range for all laboratories, (ii) the all laboratory-trimmed range for all laboratories, (iii) the sample summaries supplied back to the laboratories contained the following information: (i) the all laboratory-trimmed mean (ALTM) and range for all laboratories, (ii) the all laboratory-trimmed range for all laboratories, (iii) the all laboratory-trimmed mean (ALTM) and range for all laboratories, (iv) the all laboratory-trimmed range for all laboratories, (v) the all laboratory-trimmed mean (ALTM) and range for all laboratories, and (vi) the all laboratory-trimmed range for all laboratories.

**UPTAKE OF WHO5 METHODOLOGY**

In 2009-2010, prior to the release of WHO5, there was a wide range of classification criteria in use including Strict criteria and those described in WHO manuals going back to 1987. However, the criteria defined in the WHO5 and using a lower reference limit of 4% normal forms were adopted by 50% of laboratories in the first two years following its initial publication and went up to 85% in five years, confirming a major effort by laboratories to update their protocols to use the most recent WHO5 manual. This was mainly at the expense of the use of the WHO4 dropping from 61% to 8% in the five years since the introduction of WHO5. By 2018-2019, more than 90% of laboratories were using the one classification system of the WHO5.

In summary, the introduction of WHO5 resulted in the effective adoption of the its morphology classification system, laboratories showed improved between-laboratory variation over time, and the identification of normal forms over time was inconsistent as laboratories became more strict, and the instability in reporting proportions of normal forms over time is reflected in a reduction at each distribution in coefficient of variation (%). Mean normal forms reported when plotted against distribution number (n=0.7279, p=0.0001).