In this issue of Human Reproduction, Auger et al. (2016) examine sperm abnormalities in fertile and infertile men, and those with testicular cancer, extending what was published in the most recent version of the WHO Laboratory Manual for the Examination and Processing of Human Semen (World Health Organization, 2010) on normal sperm forms. That publication attempted for the first time to raise the quality of semen analysis to that accepted in other branches of medicine for clinical measurements, by providing reference values and threshold limits for semen parameter values (Cooper et al., 2010). The reference population chosen was fertile men for several reasons, not the least of which was that a man’s fertility can be precisely defined in terms of time to pregnancy of his partner after unprotected intercourse. For this population a lower limit of values of parameters considered to be essential for fertility were generated, below which values are consistent with the provider’s being from another, most likely infertile, population. As higher values of these parameters are unlikely to be associated with infertility, the fifth centile was chosen as the one-sided lower limit of the 95% confidence interval.

Sperm morphology has been one of the parameters traditionally accepted to be associated with male fertility, but the difficulty in recognizing a ‘potentially fertilizing’ (‘normal’, ‘typical’ or ‘perfect’) spermatozoon has taxed the mind of many over the years (Brazil, 2010), as has been getting agreement in the assessment of such sperm cells by technicians within a laboratory and between institutes (Pacey, 2010). For the WHO manual, the decision to accept ‘normal’ forms as those to be assessed was based on the studies showing (i) that the presence of a homogeneous subpopulation of spermatozoa of similar appearance was associated with fertility in vivo and in vitro, and these were considered to be those ‘typical’ of fertility, or ‘normal’ forms (Menkveld, 2010); (ii) that it is possible to get agreement in assessment of ‘normal’ forms by technicians if rigorous quality control and assurance processes are implemented (Pacey, 2010); and (iii) that the lower limit should allow the possible prediction of fertility.

However, in a special issue of the Asian Journal of Andrology presenting comments and dissenting views on the most recent WHO manual (Handelsman and Cooper, 2010), two criticisms of the usefulness of assessing ‘normal’ sperm forms were made. One was that the extremely low percentage of such forms as the normal lower limit (4%) in fertile men’s semen made it impossible to separate fertile from infertile men (Auger, 2010; Eliasson, 2010; Skakkebaek, 2010). The low percentage of such ‘normal’ forms resulted from the adoption of a very critical classification of forms, by rejecting borderline cases as abnormal (Menkveld, 2010). A return to the more relaxed approach (borderline forms accepted as normal), as used in earlier WHO manuals, was urged by some (Auger, 2010; Eliasson, 2010; Skakkebaek, 2010), as it should provide greater values and an expected higher possibility of distinguishing fertile from infertile men. A second criticism was the decision to ignore completely abnormal sperm forms, by those (Amann, 2010; Auger, 2010; Eliasson, 2010) who argued that these forms could provide information on the nature of the infertility (e.g. testicular function). However, agreement between technicians in the assessment of each of the many abnormal sperm forms (e.g. ‘cigar-shaped’, ‘pyriform’, ‘amorphous’), similar to that shown for ‘normal forms’, had not then, and still has not, been demonstrated. To some extent the present article (Auger et al., 2016) fills this gap.

Auger and colleagues have studied the morphology of seminal spermatozoa from 926 fertile men, defined as per WHO (2010) [excluding the morphological criterion], 1747 infertile men and 273 testicular cancer patients. By using standard seminal smear-staining procedures, the recognition of ‘normal’ forms (differing from WHO (2010) in that borderline cases were included in the classification) and 15 defined abnormal forms (Auger et al., 2010), highly experienced and quality control–proven technicians at one centre estimated the percentages of each form in semen from the three categories of men. As in WHO (2010) they provided the fifth centile of normal forms from fertile men as the lower limit cut-off, which at the higher value of 23% reflects the less strict categorization or normal forms used. They also demonstrated that there were more abnormal sperm heads than abnormal tails in fertile men—the abnormality being of texture and outline rather than size—and that generally only two abnormalities per sperm cell were found per fertile men. As expected, the infertile men and cancer patients had lower percentages of normal forms than the fertile men, and higher percentages of abnormal forms, but the cancer patients had higher percentages of micro- and macro-cephalic forms, and of spermatozoa with acrosomal abnormalities and excess residual cytoplasm, than the infertile men. There were also more defects per abnormal sperm cell in these groups than in the fertile controls.
Certain abnormal sperm morphologies showed different distributions between the three groups (e.g., acrosomal and post-acrosomal regions, bent/misaligned midpieces, multiple anomalies index), but this was also true of the normal forms; nevertheless, from the overlap of the distributions no clear-cut separation of the three groups was possible. The critical and important finding here, however, is that although the percentage of abnormal forms per se was not of diagnostic importance, the different levels of abnormality were to an extent discriminatory. Thus, from the abnormal forms Auger et al. defined three reference limits on the basis of the extent of abnormality: low level abnormality (LLA), at or below the 95th centile of abnormal forms in fertile men, representing ‘background noise’ of the spermatogenetic process; high level abnormality (HLA), at or above the 99th centile of abnormal forms in infertile men, representing an extreme level of abnormality, possibly of specific origin (genetic or environmental) and with pathological implications; and an intermediate level abnormality, between the LLA and HLA limits, representing non-random influences on sperm morphology. From such an approach different morphological attributes could be plotted for men of different seminal quality, from which emerged distinct and characteristic profiles capable not only of separating fertile from infertile men, but also of distinguishing, e.g. globozoospermia-like and multiple-head semen samples from others. This approach is definitely an advance on just assessing what is judged to be normal or abnormal forms, and would be even more informative when coupled with genetic analyses of infertility.

Because of the difficulty of the assessment, and the time required to train technicians sufficiently for appropriate agreement on it, the authors raised the question of the reasonableness of systematically determining the morphological abnormalities in all cases. Given that it was the changes in the outline, rather than length and width, of the sperm heads of the abnormal forms in fertile men, and that computer-aided sperm morphology analysis systems can determine sperm head dimensions including perimeters (Bellastella et al., 2010), the application of this methodology to automate the assessment of abnormal sperm cells may be worth considering. Furthermore, as the processes of air-drying and staining are known to introduce morphological artefacts in spermatozoa, particularly sperm heads (Yeung et al., 1997), the use of fixation- and stain-free methods of analysis, such as that of unstained living cells (Soler et al., 2014), may be useful in future studies. Lastly, just as the total number of motile and morphologically ‘normal’ spermatozoa per ejaculate is more important than their percentages in providing a minimum number of such cells compatible with fertility (WHO, 2010), it may be worth gathering data with this method on the total number per ejaculate of each of the abnormal sperm forms, to provide a maximum number associated with infertility.

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**References**


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**Author’s role**

T.G.C. wrote and approved the final manuscript.