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Editorial

Foreword to *Semen Analysis in 21st Century Medicine* special issue in *Asian Journal of Andrology*

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Semen analysis as an integral part of infertility investigations has a surprisingly long history, emerging only slowly, from under a cloud of disrepute and occupying a solitary niche outside conventional pathology tests, until relatively recently. From origins in the 19th century when spermatozoa were only identified as present or absent in cervical mucus samples from postcoital tests, even then a practice deemed "… dabbling…. incompatible with decency and self-respect…" (cited in [1]). Only in 1929 was the first paper using quantitative methods, recognisable forerunners of modern semen analysis, reported in a respectable medical journal [2]. The clinical perspective on systematic investigation of male infertility with an appropriate focus on semen analysis was first comprehensively outlined in Robert Hotchkiss's 1944 book "Fertility in Men" [1]. The systematisation of laboratory semen analysis largely stems from work Dr John MacLeod, a Scot working in New York, who staked out the ground by publishing a series of landmark papers in semen analysis over 4 decades from the early 1940s including, together with a statistician (Ruth Gold), the first large scale normative studies of semen analysis. During the post-WWII decades semen analysis became integrated into routine clinical infertility practice, typically undertaken in specialised laboratories as an adjunct to infertility clinics and performed by a small corps of laboratory scientists who focused exclusively on semen analysis and operating largely outside conventional pathology circles. In the absence of any agency taking responsibility to maintain congruence of laboratory techniques, this inevitably led to unintended divergence of customary practice in methodology and reporting.

The next development was prompted by the world-changing advent of oral contraception in the 1960s which inspired the rapidly growing World Health Organizazion (WHO) to develop its Human Reproduction Unit (1965) which evolved into its ongoing Human Reproduction Programme (HRP, 1972) with the announcement of eight Task Forces aiming to develop newer forms of contraception [3] but which overlooked male contraception. This oversight was rectified by the inauguration of the Male Task Force (MTF), the history of which was written by the late Dr Geoff Waites in his last paper [4]. Orignally chaired by Dr Alvin Paulsen, whose recent passing is sadly noted, the prime objective of the MTF was to develop practical forms of male contraception. Operating in the milieu of WHO's various political mandates, this required extending the capabilities to conduct male contraceptive studies to developing countries where clinical andrology expertise and semen analysis facilities, if they existed, were ad hoc and bore little resemblance to the methods evolved by Hotchkiss, MacLeod and their successors. In this context, Geoff Waites saw the need, and persuaded HRP to publish the 1st edition of WHO Semen Manual in 1980 which was intended not only to facilitate male contraceptive studies, but also to improve and standardise male infertility investigations, which he understood to be as inseparable as two sides of the same coin – the ability to choose the timing and size of a small healthy family. Under Geoff's outstanding and resourceful editorship, the WHO Manual went through four editions to become the WHO's most successful and popular publication as well as setting the well accepted world-wide standard for laboratory semen analysis methods ever since. Despite a hiatus of 2 years (1980–1982), during which the regressive forces that had overlooked male contraception in the first place again prevailed in suspending the MTF, Geoff Waites managed remarkably to maintain the momentum in both the MTF and its WHO Semen Manual and when required to navigate skilfully the inertia and hostility of the WHO bureaucracy.

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The imminent appearance of the 5th edition of the WHO Laboratory Manual for the Examination and Preparation of Human Semen (2010), and a related manuscript providing for the first time reference values for semen parameters, provides an excellent opportunity to discuss the relevance of these publications for Andrology in the 21st century. Through its four editions, the WHO Semen Manual has represented a wide scientific consensus among peers, a difficult feat in any field of medical science. Although in nearly all areas the 5th edition has readily maintained the agreeable consensus among the scientists comprising the editorial committee, chaired by Trevor Cooper as Geoff Waites's successor, inevitably in a few areas, divergent views generated more heat than light. In particular, the lack of population reference ranges for semen analysis (and how to obtain them) and the morphological methodology have been most troublesome over recent editions. Recognising the intelligent compromises, large and small, achieved between occasionally diametrically opposite viewpoints of experienced scientists, the guest editors of this special issue sought to make a virtue of necessity by fruitfully bringing to the surface such underlying and sometimes unresolved dilemmas. Rather than burying these untidy disagreements, we aimed to expose the seams which, though it may offend more delicate souls, is an integral part of the unending creative process of empirical science. We hope the present special issue, co-edited by a pair of admirers, colleagues and friends of the late great Geoff Waites, to whom the new edition is fittingly dedicated, and which we feel sure he would have encouraged, will be accepted in that spirit.

We invited widely experienced semen laboratory scientists to comment on pre-publication versions of the new edition of the WHO Manual and the manuscript presenting reference values, with regard to modern semen analysis methodology (Aitken [5], Amann [6], Auger [7], Björndahl [8], Brazil [9], Eliasson [10], Ford [11], Jequier [12], Lamb [13], Menkveld [14], Pacey [15]); a scientist familiar with the modern development of reference values in pathology tests (Boyd [16]) and medical scientists familiar with using semen analysis in population or clinical research studies (Bonde [17], Joffe [18], Skakkebaek [19]). Some of these contentious issues in the methodology of semen analysis and its applications, which we hoped them to highlight, are outlined below.

A major advance in this new edition of the WHO Manual, resolving the most salient critique of previous editions, is the development of the first well defined reference ranges for semen analysis. The details have been published [20] separately but these data include relatively large samples of recently fertile men as well as unselected men, forming the best representation of the general male population feasible. The former is important for diagnosis and prognosis in male infertility practice whereas the latter is necessary for population studies using semen analysis as a surrogate variable for male fertility. The determination of reference intervals to provide population-based reference ranges is widespread in clinical chemistry [21]. It is common for the central 95% of values in the reference distribution to constitute the reference range, the upper and lower reference limits defining what can be considered normal. But for semen analysis should this range span a double-sided, as typical for most pathology tests, or a one-sided reference interval? Another option is the use of decision limits set at different levels from population evidence-based levels, as has been done for serum cholesterol on the basis of large scale primary prevention trials which dictate healthy levels that differ systematically in being lower from extant population levels for a common disorder. Whether this decision limit approach is applicable to semen analysis revolves around the scope of effective treatments but the dearth of effective treatment for infertile men with oligozoospermia makes such application seem dubious in the immediate future. Nevertheless, the availability of high quality reference data provides a concrete basis for such debates which formely centred on simplistic arguments about whether these limits were too high or low.

Categorising samples as under or above the reference limits may be technically easy but interpreting the categories is not. Firstly, such thresholds described by evidence-based 95% confidence intervals, are empirical and not immutable. Second, by definition, 5% of the reference population lies outside the reference limits, so that a sample's falling outside these limits may indicate merely a statistical extreme, but not necessarily genuine pathological or biological abnormality which has no necessary linkage to the Fisherian 95% convention. Some authors discredit the whole concept of dichotomising continuous variables and stress assessing merely the sample value obtained [22]. However reference values also serve as decision criteria for clinical management, and for population studies, whether recognised or not. There remains widespread ambiguity in interpreting reference limits and their utility.

Which reporting end-points for sperm output should be sought in a semen analysis? The standard variables have been semen volume, sperm concentration (allowing a multiplication to derive the total number of spermatozoa per ejaculate) together with sperm motility and sperm morphology (the latter as "functional" aspects of the cells). Should sperm concentration be replaced by total sperm number per ejaculate as a better reflection of average testicular sperm production and of what is present at the site of fertilisation in the female reproductive tract? Does the time-dependent dilution by accessory gland fluids provide any biologically meaningful information? Divergence between sperm concentration or total sperm number may provide conflicting information if the semen volume is the primary variable changing (e.g. with age [23]). Should the numbers of progressively motile or morphologically normal spermatozoa be even better measures?

Quality assurance, now mandatory for virtually all laboratory measurements, is being introduced into the andrology laboratory. Yet, remarkably, a recent debate among andrologists found voices against its introduction [24, 25] on the basis of excessive cost and effort for the value of the returns. Is this retrogressive conservatism or enlightened appraisal of a paltry addition to reported results?

The reporting of round cell or leukocyte number has been debated with respect to the validity of the 1×10^6 mL^{-1} cut-off. The current standard method (1:10 dilution of semen) for a round cell/peroxidase test at the cut-off concentration will produce only 10 spermatozoa per central square (100 nL) of a haematocytometer chamber. Could all published values of round cells in semen be wrong?

Should motility be replaced by the more objective velocity of progressive spermatozoa and be assessed by computerised systems?

How should sperm morphology be assessed? Is the pursuit of the perfect spermatozoon, based loosely on the subpopulation of potentially fertilising spermatozoa arriving close to the site of fertilisation, an improvement over the previous system of assessing all abnormal forms? The difficulty in getting technicians to agree on a well-defined "normal" head form makes widening this to include agreement on "amorphous", "tapered", "pyriform" and other forms a bold task and requires a leap of faith in subjective human assessment. The use of computerised systems to assess sperm heads objectively is an attractive idea but the reality of bias introduced by selection of analysable images, adequate staining, correct digitisation, among other factors, does not meet this ideal.

What does vitality mean for a spermatozoon: can the mere exclusion of an impermeant dye by the membrane of a particular organelle provide information of the spermatozoon's viability? Acrosome-reacted spermatozoa have lost their anterior plasma membrane and take up membrane-impermeant propidium iodide [26]; according to vital dye staining such fertilising cells are considered non-viable—is it meaningful to judge such functionally competent cells as non-viable?

Many tests of sperm function (for assessing cumulus penetration, zona binding, chromatin and DNA structure) and genomic potential (histone methylation, RNA transcripts) have been developed [27] but none is routinely employed by andrology laboratories. Some require expensive equipment or material not widely available. However, centralised processing of these tests in accredited laboratories could be introduced if they were shown to be useful. If intra cytoplasmic sperm injection (ICSI) is only recommended when sperm damage is so extensive that IUI and *in vitro* fertilization (IVF) are futile, do we select for abnormal embryos by deliberately introducing highly damaged spermatozoa? In these days of ICSI, where tests of the fertilising spermatozoon cannot be done, is it worth doing sperm function tests at all?

Finally, the last decade and a half have seen Andrology's most prominent public profile to be dominated by the alarming claims that sperm counts are falling throughout the world as a result of estrogenic pollution. This kind of apocalyptic alarmism, relating directly to sexual (reproductive) function, was a heaven-sent blessing for media whose commercial product is sensationalist attention-seeking as a vehicle to help sell their advertising. For such a valuable commodity, the media form a vast echo chamber turning tentative musings into endlessly repeated and increasingly exaggerated claims, ultimately distracting and derailing proper scientific testing of what was an implausible hypothesis. The editors therefore invited the key proponents of the claims that estrogenic pollution causes decreases in human sperm output Drs Skakkabaek [19] and Sharpe each to summarise their experience of the lessons learned arising from this hypothesis.

This brief and provocative overview of the many problems inherent in semen analysis and its interpretation as done today, and the changes touched upon in the new manual, set the scene for the articles in this supplement. We were pleased that all but one writer who hold conflicting views on these issues, accepted the invitation to contribute to this Special Issue. We are fortunate indeed that these bold reviewers have written unashamedly honest appraisals on the topics we had chosen for them.

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