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Foreword

Computer-assisted sperm analysis and reproductive science; a gift for understanding gamete biology from multidisciplinary perspectives

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The science of reproductive biology has been a source of fascination for centuries and has been marked both by important landmarks of discovery and influential, but persistent, errors of fact. The first century physician and philosopher, Galen of Pergamum, carried out empirical investigations on both humans and animals and proposed that conception required a combination of both male and female 'principles'. He recognised the importance of male's semen for conception, despite the views propounded by others that females were not particularly relevant in what we now understand to be a 'genetic' sense. Having dissected female reproductive tracts Galen suggested that the ovaries were analogous to the testes, and therefore capable of producing 'female semen', which was visible in the uterine horns and Fallopian tubes (for a review of these early investigations and the disagreements between Galen and Aristotle, see Connell (2000)). More than a thousand years of technological developments finally resulted in the invention of lenses that could enable biologists to examine males' semen and observe the strange 'animalcules' that we now recognise as spermatozoa (Gonzalès 2006; Gest 2009; Karamanou et al. 2010). It seems obvious to present day scientists that these cells would be essential for conception, but it took almost two more centuries before the role of spermatozoa was recognised in the late 1830s (Ribatti 2018).

Once the role of spermatozoa in reproduction was widely recognised, they became a source of research interest in many different fields. Their ability to swim encouraged the rather over-simplified, but easily understood, belief that sperm swimming speed must reflect their potential fertility. In turn, this led to the development of methods for assessing and measuring sperm swimming speed. At first, these methods were subjective and involved assigning simple scores, typically using a scale of 1–5, where a score of 5 meant very high levels of activity. This approach was widely used by scientists involved in the initial stages of developing sperm cryopreservation and artificial insemination methods (Emmens and Swyer 1948; Emmens and Blackshaw 1950; Blackshaw and Emmens 1951), and in combination with advances in statistical methods, such as analysis of variance and the randomisation of experimental treatments, produced significant outcomes upon which much of the agricultural industry is still based. More recently, evolutionary biologists appreciated the significance of sperm production, morphology and motility and, with input from mathematicians and theoretical biologists (Parker 1970; Parker 1982), they developed what is now the very lively field of sperm competition research. This field has attracted scientists who are interested in studying many different species, as diverse as insects, birds, fishes and mammals. Several of the papers in this special issue reflect the current interests of researchers working with sperm assessment in various species (Van der Horst et al. 2018*a*, 2018*b*; Yániz et al. 2018*a*). In the 1970s and 1980s, developments in computer and imaging technologies began impacting sperm research and it became possible to track the progress of individual spermatozoa across a field of vision, capturing data that could be used for further detailed analysis. These advances suddenly began to produce very large datasets, whose significance was largely rather opaque because of the inherent heterogeneity within semen samples. Untangling such data by the use of multivariate statistics then became very important, and is a topic of continued interest as evidenced in several of the papers within this special issue (Ramón and Martínez-Pastor 2018; Yániz et al. 2018*b*).

Given the availability of appropriate technologies, it was relatively easy for computer systems to measure the characteristics of sperm tracks, but routine clinical and agricultural laboratories were generally more interested in obtaining accurate assessments of sperm concentration. They were also hoping to distinguish those individual semen samples that might be regarded as subfertile or infertile. These have been, and still remain, rather challenging problems. Assessing human sperm concentration in clinical laboratories has been

fraught with technical difficulties; some of these points are addressed by various authors within this special issue. One such article (Tomlinson and Naeem 2018) focuses on the practical integration of computerised semen analysis into routine clinical laboratory practice, something that has been difficult to accomplish. It has often seemed that, while clinical laboratories perform a basic range of tests on semen samples, they do not really know how to interpret the outcomes in terms of patients and their likelihood of conception. This conundrum, which involves subjective attitudes as well as objective techniques, is discussed in a paper by Gallagher et al. (2018). Other authors within this issue have examined the potential for inaccuracies arising from the design and use of different chambers for sperm sampling (Bompart et al. 2018; Soler et al. 2018; Yeste et al. 2018).

Addressing, or coping with, species diversity using CASA-Mot requires considerable biological as well as technical knowledge. Sperm activation mechanisms differ widely among species, and are nowhere more apparent than with freshwater fishes. These spermatozoa are essentially inactive within the male reproductive tract but, as soon as they meet the hypo-osmotic environment of freshwater, they exhibit almost explosive activation of motility; however, it may only last a few seconds or minutes. The sperm plasma membranes are inevitably damaged, but motility activation is caused by rapid changes in protein phosphorylation status. This poses an unusual problem for anyone interested in the measurement of fish sperm motility – i.e. how to cope with these time-related changes? These are important questions and we have therefore included contributions about fish spermatozoa from three different laboratories (Boryshpolets et al. 2018; Caldeira et al. 2018; Gallego and Asturiano 2018).

Sperm activation is not, however, a property unique to fish sperm. It may be less noticeable when dealing with mammalian spermatozoa, but careful investigations of mouse, boar and even human spermatozoa have established that these cells are rapidly activated when they are exposed to bicarbonate ions (Wennemuth et al. 2003). We now know that the mammalian female reproductive tract exploits this mechanism for controlling sperm transport and storage (Holt and Fazeli 2016), but this is also highly relevant when using CASA-Mot. It emphasises the critical importance of the nature of sperm suspension media in attempts to compare data between species, or even between laboratories. We have included one paper to emphasise the importance, and even the exploitation, of mammalian sperm activation in research (Holt and Satake 2018).

Instrumentation for CASA analysis has developed rapidly alongside our biological understanding, so we wanted to reflect some of this progress. One of the papers (David et al. 2018) is interesting because it capitalises on some of the earliest observations made with ram semen; namely, that if an undiluted sample is viewed through a low powered objective, it is usually possible to see a great deal of swirling and wave motion. Although the early pioneers of sperm assessment developed rating systems to rank such sperm behaviour, a group of biologists and mathematicians has developed an unusual CASA approach that quantifies the wave motion to such a degree of accuracy that the data are predictive of AI success in sheep. This approach is probably not possible with other species, partly because they rarely display the same type of wave motion, but also because the sheep data analysis is reliant on comparison with a large dataset relating fertility with sperm analysis that has been amassed over several years. Microscopy for CASA itself may eventually be changed altogether, as traditional glass lenses themselves are phased out in favour of lenseless microscopy.

The editors are very grateful to Dr Trevor Cooper who made the initial suggestion that we have now reached a point in the development of CASA-based techniques and science that a special issue would be worth developing. We would also like to thank all of our contributors, none of whom hesitated when we asked them to write one of the papers, and all of whom have collectively produced such an exciting and informative set of articles. Finally, we thank the editor of *Reproduction Fertility and Development*, Graeme Martin, and his editorial colleagues, for their unfailing support and encouragement throughout the process of putting this special issue together.

A note on CASA terminology

In preparing this special issue, and an earlier special issue published in 2016 by *Asian Journal of Andrology*, it became apparent that the conventional CASA terminology was inadequate to describe the different uses to which the technology is now being put. In the papers initially submitted, authors used several acronyms to describe the method they were using, including CASMA (Computer-Aided Sperm Morphology Analysis), CASMA-F (when fluorescent dyes were assessed) and ASMA (Automated Sperm Morphology Analysis). With these terms, neither the nature of the automation (with ASMA) nor the morphology examined (with CASMA-F) is clear from the abbreviation. In this special issue, for example, for spatulate spermatozoa, the sperm head itself, its acrosome, or its nucleus can each be analysed by the system, and filiform spermatozoa permit additional values on the length of the head and tail. Soler et al. (2016) proposed that we need a change in terminology to one that indicates which sperm feature is being measured by the system. The acronym CASA itself computer-aided/assisted sperm analysis) is uninformative since the analysis could refer to any aspect of spermatozoa: concentration, motility, kinematic parameters or morphology, or combinations of these variables. Indeed, the early papers used this blanket term to cover them all although the term CASA today is generally used in association with sperm kinematics.

For this special issue, we have therefore consistently used the following hyphenated compound terminology as suggested by Soler et al. (2016): the generic use of CASA for any kind of sperm computer-aided sperm analysis, followed by an abbreviation indicating the analysis performed, i.e. CASA-Conc (for concentration), CASA-Mot (for motility, including kinematics), CASA-Morph (for morphology, including morphometry) and CASA-DNA (when DNA is being studied). These could be extended if necessary to indicate when fluorescent dyes are used for morphology CASA-Morph-F) or when DNA fragmentation is being assessed (CASA-DNAf).

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