Introduction
While bulls which fail the breeding soundness evaluation (BSE) are almost certain to be sub- or infertile, for a variety of reasons passing the BSE does not guarantee an acceptable level of fertility. The ability of spermatozoa to produce energy for motility, antioxidants for cellular defence, to undergo modifications essential for fertilization to occur and to recognise and activate the egg to produce an embryo all require the presence of functional biochemical pathways and proteins which may be ascertained using emerging analytical techniques. The aim of this paper is to highlight the possible applications of more advanced molecular and biochemical sperm assessments that may be implemented in a clinical setting to facilitate interventions for fertility rescue based on early indications of cellular stress. The development of on-farm assays to measure these factors may be added to the toolbox for a more comprehensive BSE of the future.

Reactive oxygen species: friend or foe?
Conventional sperm analyses such as subjective motility, viability, morphology and cell concentration will always be the first port of call for the diagnosis of infertility in a clinical setting. However, such analyses do not provide information about impaired subcellular processes in spermatozoa, and defined pathophysiological diagnosis of male infertility is often overlooked. Like all cells of the body, sperm require oxygen to produce energy via oxidative phosphorylation (OXPHOS). While the sperm of most species utilise OXPHOS only sparingly, bull sperm depend almost entirely on OXPHOS for the production of energy to maintain life, resulting in the formation of excess reactive oxygen species (ROS) which can either be measured directly or through the assessment of the downstream products of ROS-induced damage; an approach which can provide more information about the state of the system as a whole. These downstream markers can be quantified using various assessments of membrane damage, residual antioxidant capacity, DNA damage and apoptosis, all of which can be accurately measured using flow cytometry, although some assays may be performed via microscopy.

Conclusion
By the time valuable bulls are diagnosed with infertility based on conventional sperm assessments (such as motility, morphology and concentration), it is often too late to intervene to prevent this demise. More advanced molecular and biochemical sperm assessments will provide further insights for clinicians into the mechanisms underpinning subfertility and infertility, facilitating early management interventions and the rescue of fertility based on indications of cellular stress prior the appearance of clinical symptoms.