Male subfertility is a very significant global problem. Epidemiological data show that approximately one in seven couples are classified as subfertile [1]. Sperm dysfunction is the single most common cause of male subfertility. An older study employing a sperm concentration cut-off of <20 × 10^9/ml found that 20% of 18-year-old men were classed as subfertile [2]. Although it is too simplistic to base a classification of subfertility solely on sperm concentration, the reported frequency of male subfertility points to a high proportion of the population being affected, compared with other prevalent diseases such as diabetes. What is more worrying is the likelihood that sperm counts are falling and the prevalence of male subfertility is increasing [3,4]. Moreover, male fertility has been shown to be a barometer of overall health and longevity [5–7], and significant evidence suggests that the health of future generations may be influenced epigenetically by the quality of their father’s spermatozoa, which may have been altered by his diet and/or lifestyle [8–10]; perhaps such effects underlie the fall in sperm counts [4]. In addition, there are many substances and products that are toxic to spermatozoa in our everyday environment [11]. Unfortunately, little progress has been made towards answering fundamental questions in andrology or in developing new diagnostic tools or alternative management strategies for infertile men other than ICSI [12,13]. A recent expert meeting highlighted evidence gaps and important research areas, and proposed a strategic approach whereby andrology might make the rapid progress necessary to address key scientific, clinical, and societal challenges that face our discipline [14]. Andrology is therefore a pivotal discipline in modern medicine, and it is against this background that we have updated this handbook.

Semen analysis provides a comprehensive view of the reproductive functioning of the male partner of the subfertile couple. It includes assessments of sperm count (which reflects sperm production, transport through the male genital tract and ejaculatory function), sperm motility (a basic functional marker of likely sperm competence), sperm vitality (to distinguish between dead spermatozoa and live, immotile spermatozoa), sperm form (aspects of sperm production and maturation), and the physical appearance of the ejaculate (semen production). In addition to this basic semen assessment there are further tests that can be performed – what we have termed extended semen analysis – permitting further analyses that assess more functional aspects of the semen sample. Such tests include biochemical examinations to evaluate the secretions from the auxiliary sex glands, the detection of anti-sperm antibodies, and the use of computer-aided sperm analysis (CASA) to examine sperm motility patterns (‘kinematics’, see Chapter 6).

A high quality, comprehensive semen assessment is not just the cornerstone of the diagnosis of male subfertility, it is also the starting point for providing prognostic information. While the basic semen assessment has been performed for over 70 years, there have been a number of studies questioning the value of the traditional semen characteristics (sperm concentration, motility and morphology) in the diagnosis and prognosis of male subfertility [15]. Partly, this is the result of an incomplete understanding of what clinical information a semen assessment can provide (see below), but primarily it is because the basic assessments are usually performed using inadequate methods with limited understanding of the technical requirements and poor quality assurance [16]. An enduring example of this is the UK survey of laboratories performing ‘andrology tests’, which showed dramatic variation from WHO recommended procedures leading to uncritical reporting of results [17,18].

In this handbook we provide a detailed, step-by-step guide using robust methods for examining human semen. We have also included a comprehensive explanation of staff training, and sections on Quality Control, Quality Assurance and Quality Improvement. Adoption of such methods and procedures will lead to a significant improvement in the quality of the data produced by an andrology
laboratory, and therefore more robust clinical information. At the time of completing this book (August 2021), during the second year of the COVID-19 pandemic, we have at last seen the convergence of basic semen analysis methodology between the ESHRE Andrology Special Interest Group (SIG) Basic Semen Analysis Course methods (this book), the recommendations of the sixth edition of the World Health Organization manual ('WHO6') [19], and the reference methods for basic human semen examination published in ISO Standard 23162:2021 [20] which should be adopted by ISO 15189-accredited medical laboratories worldwide.

One matter that has been discussed in relation to semen analysis is the number of specimens that must be analysed from each individual. Quite often at least two specimens are said to be required to get a representative result for the individual [21,22]. However, when based on laboratory data, a considerable portion of the variability in results can be ascribed to technical variability due to poor quality laboratory methods. Thus, with poor technical quality (including low numbers of spermatozoa assessed) investigations of multiple specimens from the same subject can, at least in part, compensate – but is not cost-efficient either for the patient and their partner or for the laboratory. The reason why epidemiological studies investigating men for possible reproductive toxicological effects only need to produce one specimen is most likely because the variability in individual specimens ‘disappears’ when average values are used and differences in averages between groups can be analysed [23]. Although there is a considerable biological variability in semen analysis results (see Chapter 2), especially concerning sperm concentration, the clinical evaluation of the man does not always require analyses of several ejaculates. For the primary investigation of the man in a subfertile relationship, information from the very first ‘quality’ semen analysis can be enough to direct the continued investigation – either a very poor result indicating the need for direct clinical andrological investigations, or a very good result indicating that further basic semen analyses will not reveal any more pertinent information [24–26]. In those subjects with intermediate results, valuable information can be gained from repeated semen analysis. The methods as described in this handbook are designed to minimize variability due to technical factors, and thereby optimize both the evaluation of the man and the laboratory work [27].

For the proper use of semen analysis results, appropriate interpretation is fundamental. With a few clear exceptions (e.g. azoospermia), the data cannot provide unambiguous information about the chances of future conception, either in vivo or in vitro. Currently, there is a clear tendency to over-emphasize the value of a single parameter, e.g. strict cut-offs for ‘normal’ sperm morphology as used in ART clinics to decide that ICSI is ‘necessary’. However, as has been known for seven decades, there is a considerable overlap between the semen characteristics of fertile and subfertile men, so no single parameter can be used to provide prognostic information about the fertility potential of the couple [28,29]. A combination of several variables (motility, morphology and concentration) does give more accurate diagnostic and prognostic information, although there will always be overlaps between what is considered fertile and subfertile [26,30,31]. Irrespective of the low predictive value for the reproductive success of the couple, a comprehensive semen analysis provides information about the status of the male reproductive organs, and this is important in the wellbeing of the man. The results of a semen analysis are often used as a sentinel marker for the potential treatment pathway for patients. For example, a primary question in ART clinics remains: is the semen of this man suitable for IUI, or is IVF, or even ICSI, needed? [28] Primarily, what the clinic is trying to do is determine whether there are indications that the man will have a high likelihood of failure using a particular treatment modality, i.e. the man’s spermatozoa are unsuitable for insemination by IUI, and IVF is indicated. However, despite the plethora of literature surrounding this area, there are still no simple answers. For example, a meta-analysis of the literature trying to ascertain the number of spermatozoa that have been (can be) used as a cut-off for IUI success concluded that there was no such cut-off, and that the data available were of insufficient quality to provide a robust answer [32]. Of course, the quality of the sperm preparation methodology (and also the products used, see Chapter 9) will also impact on treatment outcome, confounding any simple relationship between pre-treatment semen characteristics and treatment outcome.

For the comprehensive investigation of a man’s fertility potential, it is essential not only to perform a semen analysis, but also that a physical examination be performed and a complete medical history taken [33]. Accurate interpretation of a semen analysis cannot be made without knowing the patient’s history, and having information from a physical examination and other laboratory investigations, e.g. hormone
analyses [33]. Reduced fertility potential can be secondary to other diseases that should be properly investigated and treated; it is thus irresponsible and unethical to embark upon an infertility work-up without a complete physical examination and history [14,33].

A common source for misunderstandings and misinterpretations is the use of qualitative terms such as oligozoospermia and asthenozoospermia. Originally, these terms were used to characterize laboratory findings before the quantitative measures had become usable and reliable. But subsequently, these terms have been given precise limits on quantitative scales, creating the false impression of dichotomy (two clearly separated outcomes, like subfertile and fertile), and even a ‘diagnosis’, based on semen characteristics – as opposed to the true situation of a sliding scale between severely infertile (but not sterile) and fertile. In an effort to reduce such confusion in the future, we have abandoned the use of all such qualitative terms and urge everyone working in the field to do likewise. Just describe what you see, as objectively and quantitatively as possible, and interpret the test results within the holistic medical context for the subject, especially the particular circumstances that exist within the reproductive unit of which he is part, i.e. with the female partner, since (sub)fertility is always a feature of a couple.

References
Introduction


