Other consensus threshold values	
pH	> 7.2
Peroxidase-positive leukocytes (10^6/mL)	< 1.0
Tests for antibodies on spermatozoa	
MAR test (motile spermatozoa with bound particles, %)	No evidence-based reference values. Each laboratory should define its normal reference ranges by testing a sufficiently large number of fertile men.
Immunobead test (motile spermatozoa with bound beads, %)	No evidence-based reference limits.
Accessory gland function	
Seminal zinc (µmol/ejaculate)	≥ 2.4
Seminal fructose (µmol/ejaculate)	≥ 13
Seminal neutral α-glucosidase (mU/ejaculate)	≥ 20

CIs = confidence intervals; MAR = mixed antiglobulin reaction; NP = non-progressive; PR = progressive (a+b motility).

** Distribution of data from the population is presented with one-sided intervals (extremes of the reference population data). The lower 5th percentile represents the level under which only results from 5% of the men in the reference population were found.*

If semen analysis is normal according to WHO criteria, a single test is sufficient. If the results are abnormal on at least two tests, further andrological investigation is indicated.

None of the individual sperm parameters (e.g., concentration, morphology and motility), are diagnostic *per se* of infertility. According to WHO 5th edition reference criteria it is important to differentiate between the following [1553]:

- oligozoospermia: < 16 million sperm/mL;
- asthenozoospermia: < 32% progressive motile sperm;
- teratozoospermia: < 4% normal forms.

According to the WHO 6th edition reference criteria this subdivision is not reported, although the EAU Guidelines panel considers this further segregation still clinically relevant in the everyday clinical practice.

Often, all three anomalies occur simultaneously, which is defined as oligo-astheno-terato-zoospermia (OAT) syndrome. As in azoospermia (namely, the complete absence of spermatozoa in semen), in severe cases of oligozoospermia (spermatozoa < 5 million/mL) [1554], there is an increased incidence of obstruction of the male genital tract and genetic abnormalities. In case of azoospermia, full andrological investigation should be warranted to classify obstructive azoospermia versus non-obstructive azoospermia. A recommended method to diagnose absolute azoospermia versus cryptozoospermia is semen centrifugation at 3,000 g for 15 minutes and a thorough microscopic examination by phase contrast optics at ×200 magnification of the pellet. All samples can be stained and re-examined microscopically [1555]. This is to ensure that small quantities of sperm are detected, which may be potentially used for intra-cytoplasmic sperm injection (ICSI); therefore removing the need for surgical intervention.

Advanced examinations

Obsolete tests such as the human oocyte and human zona pellucida binding and the hamster oocyte penetration tests have been completely removed. Research tests include assessment of ROS and oxidative stress, membrane ion channels, acrosome reaction and sperm chromatin structure and stability, computer-assisted sperm analysis (CASA).

Measurement of Oxidative Stress

Oxidative stress is considered to be central in male infertility by affecting sperm quality, function, as well as the integrity of sperm [1556]. Oxidative stress may lead to sperm DNA damage and poorer DNA integrity, which are associated with poor embryo development, miscarriage and infertility [1557, 1558]. Spermatozoa are vulnerable to oxidative stress and have limited capacity to repair damaged DNA. Oxidative stress is generally associated with poor lifestyle (e.g., smoking) and environmental exposure, and therefore antioxidant regimens and lifestyle interventions may reduce the risk of DNA fragmentation and improve sperm quality [1559]. However, these data have not been supported by RCTs. Although ROS can be measured by various assays (e.g., chemiluminescence),