# Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests

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Semen analysis is the first step to identify male factor infertility. Standardized methods of semen analysis are available allowing accurate assessment of sperm quality and comparison among laboratories. Population-based reference ranges are available for standard semen and sperm parameters. Sperm numbers and morphology are associated with time to natural pregnancy, whereas sperm motility may be less predictive. Routine semen analysis does not measure the fertilizing potential of spermatozoa and the complex changes that occur in the female reproductive tract before fertilization. Whether assisted reproduction technology (ART) is required depends not only on male factors but female fecundity. Newer tests should predict the success of fertilization in vitro and the outcome of the progeny. (Fertil Steril® 2014;102:1502–7. ©2014 by American Society for Reproductive Medicine.)

**Key Words:** Sperm concentration, sperm motility, sperm morphology, reference ranges, female fecundity

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Semen analysis is the cornerstone for the assessment of the male partner in a subfertile couple. Compared with many other tests used in the assessment of the infertile couple, semen analysis has been standardized throughout the world. This was made possible through the efforts of the World Health Organization (WHO) since the 1970s by producing, editing, updating, and disseminating a semen analysis manual (1). The manual provides step by step methods on how to perform a routine semen analysis, guidance on establishing internal and external quality control for these measures, and recommendations on more commonly used tests to assess sperm function. The goal of the manual is to improve the standards of semen analysis and to ensure that the semen and sperm parameters assessed in one laboratory using this manual will be the same as the analysis done in another laboratory using the same manual. International and national societies of andrology, reproductive medicine, human reproduction, and pathology contributed by providing hands on training to ensure that the technologists are using these standardized methods to assess semen and sperm quality. This allows comparative studies and pooling of data from across the globe for epidemiology studies to assess semen quality (2, 3) and to develop reference ranges for semen and sperm parameters (4). Semen analysis should be performed in laboratories with experienced technologists who have been trained in these standardized methods for routine clinical examination of semen. Despite our ability to assess sperm quality through a semen analysis methodology harmonized across laboratories, the use of these parameters cannot precisely and accurately predict the fertility of a man presenting to a clinician. This is because there are many factors in addition to sperm and semen quality that contribute to the ability of spermatozoa to fertilize an oocyte. To reach and fertilize the oocyte ejaculated spermatozoa have to traverse the female reproductive tract, hyperactivate and undergo acrosome reaction at the correct time and site, penetrate the cumulus and zona pellucida (ZP), and ultimately fuse

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with and fertilize the oocyte. The assessment of some of these changes in the spermatozoa will be discussed in other articles in this series. In addition to sperm function, female factors are extremely important to ensure optimization of the condition of the oocyte to allow for fertilization (5).

## DEVELOPMENT OF REFERENCE RANGES FOR SEMEN QUALITY AND SPERM PARAMETERS

Many studies have been criticized for the selection of subjects and methods used to develop reference ranges for semen and sperm quality, in particular the thresholds defining male factor subfertility using sperm concentration, motility, and morphology, the three classic sperm parameters measured by all laboratories. The WHO initially adopted a sperm concentration of <20 million/mL, >50% motile, and normal sperm morphology of >50% as thresholds below which subfertility may be present. This was based on studies done in the 1950s by Macleod and colleagues (6–9) in 1,000 men of known fertility and 1,000 couples with subfertility. More recent studies, in 2001, evaluating male partners of fertile and infertile couples suggested that lower thresholds of sperm concentration <13.6 million/mL, motility <32%, and normal morphology <9% should be used to define possible male factor infertility (10). The WHO collected data from >4,500 men in 14 countries, including prospective and retrospective studies on fertile men and men of unknown fertility. It is important to note that all the centers used the WHO manual for semen and sperm analyses. Data from men with proven fertility whose partners had a time-to-pregnancy of <12 months were then chosen to provide reference ranges for semen parameters (4). Using a one-sided lower reference limit of the 5th percentile (95% confidence intervals [CI]) the lower thresholds for semen parameters are as follows: semen volume 1.5 mL (range, 1.4–1.7 mL); sperm concentration 15 million/mL (range, 12–16 million/mL); total sperm number per ejaculate 39 million (range, 33–46 million); sperm motility 40% (range, 39%–42%); sperm morphology using strict criteria 4% normal forms (range, 3%–4%); and vitality 58% (range, 55%–63%). The semen quality from the general population was lower than that of fertile men. The WHO recommends using these reference limits in conjunction with clinical assessment, including the female partner's fecundity, to determine the fertility prospects for the couple.

## SEMINAL FLUID: COLLECTION AND ASSESSMENT

The seminal fluid is made up of a mixture of secretions from the testis, epididymis, prostate, and seminal vesicles, and the contribution from each of these glands varies by the interval of abstinence and the method used to obtain the semen samples. Although sexual abstinence of 2–7 days is generally advised before submission of a sample for analysis (1), a recent study suggests that in subfertile men, the samples should be collected after 1 day of sexual abstinence for optimal semen quality (11). In men, semen samples collected by masturbation in the clinic may be of a lower quality than those collected at home (12); however, erotic materials or isotonic lubricants do not appear to influence the quality of the sample (13, 14).

When seminal fluid volume is markedly reduced, the clinician should suspect incomplete collection, severe androgen deficiency and obstruction in ejaculatory ducts, or bilateral absence of the vas deference. In the latter two conditions, the seminal fluid will have an acidic pH, very low fructose levels, and no spermatozoa, and the diagnosis can be confirmed by physical examination, confirming bilateral absence of vas deferens or by transrectal ultrasound showing dilated seminal vesicles in ejaculatory duct obstruction (15). There are a number of biochemical tests to measure functions of the accessory gland including zinc and acid phosphatase (prostate), fructose (seminal vesicle), and carnitine and alpha-glucosidase (epididymis) (1). These biochemical tests are not routinely performed and are of rare clinical usefulness as biomarkers of male factor infertility.

## SPERM CONCENTRATION AND TOTAL SPERM NUMBER IN THE EJACULATE

The standardization of measurement of sperm concentration and semen volume allows for a more accurate calculation of sperm output. Despite many comments and discussions about using sperm concentration as a biomarker of male factor infertility, the accurate assessment of number of spermatozoa in an ejaculate remained the standard practice for the evaluation of the infertile couple. Fundamentally a single parameter cannot be used as a valid biomarker of fertility because a multitude of factors contribute to infertility including the inherent biological variability of sperm concentration, the methods of fertilization (in vitro vs. in vivo), the health of the man at time of collection, and female factors. Sperm concentration in a man showed considerable variation and at least two semen samples should be examined before providing a conclusion that the sperm concentration or total sperm count is below the reference range (16). Retrospective data analyses from cryobanks on 18–20 consecutive semen samples from 48 semen donors showed that an optimal duration of abstinence to distinguish high or low sperm production may be between 42 and 54 hours and collection of three samples may provide results closer to the true value (17). Amann (18, 19) also suggested that the rate of daily sperm production may better reflect altered spermatogenesis and that the assessment of total number of spermatozoa per ejaculate is reflective of sperm production provided the abstinence interval is appropriate.

The lower limits of sperm concentration and total number of sperm per ejaculate that reflects male subfertility is not known. The 5th percentile of WHO reference value for sperm concentration is 15 million/mL (95% CI 12–16) and for total sperm number per ejaculate is 39 million/mL (95% CI 33–46). This is based on data generated from 1,859 fertile men with a time-to-pregnancy of less than 12 month (4) using a one-side distribution as there is no upper limit of sperm concentration that is associated with infertility. It is now recognized that there are geographic differences in sperm concentration across countries. Epidemiological studies (3, 20–22) suggest that this may be related to environmental