Chapter 22
How does semen analysis assist in understanding the reproductive status of the male?

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What composes semen?

The ejaculate consists of spermatozoa (sperm) in a mixture of secretions mainly from the prostate gland (Chapter 20) and seminal vesicles (Chapter 21) with small contributions from the epididymides (Chapter 18). The contribution from each of these glands varies by species and depends on the interval of abstinence and the method used to obtain the semen samples. In men, about 90% of volume of the seminal fluid is composed of secretions of from the prostate and seminal vesicles. There is some evidence that the total volume of the ejaculate collected in a clinic may be lower than that during sexual intercourse. As the volume of the seminal fluid may be quite variable, the total number of spermatozoa in the ejaculate may be a more important parameter than the sperm concentration in the ejaculate.

The structure of the human spermatozoon is shown in Fig. 1. Enclosed in a plasma membrane, the spermatozoon consists of the head which has the nucleus (chromatin containing the genetic material and epigenetic elements) covered by the acrosome. The flagellum (tail) has a mid-piece, principal piece, and end piece. The principal piece contains nine microtubule doublets plus a central doublet forming the axoneme which is surrounded by outer dense fibers enclosed by the fibrous sheath. The midpiece contains mitochondria wrapped around dense fibers and axoneme. The end piece has lost the axoneme structure as well as the outer dense fibers and fibrous sheath. The flagellum is essential for progressive forward movement of the spermatozoa. The shape and size of the sperm differ in different species as shown in Fig. 2.

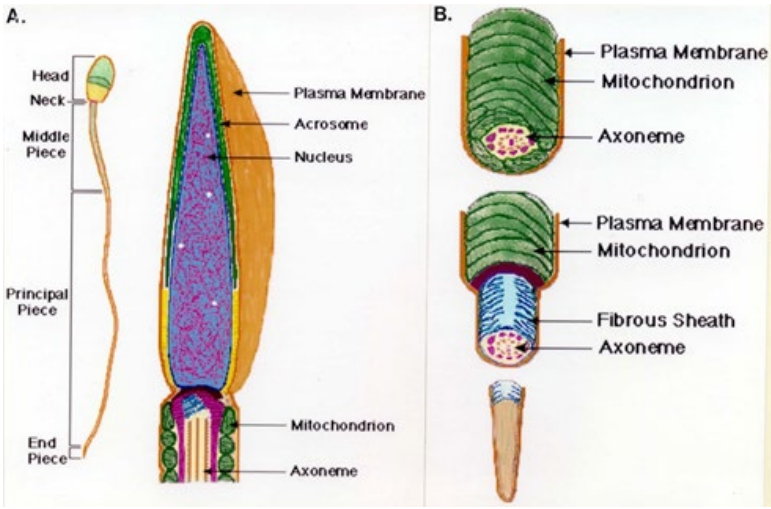

Figure 1. A) Major elements of a human spermatozoon that are common to mammalian species. **B**) Middle piece (top), principal piece (middle), and end piece (bottom) of a spermatozoon viewed in cross-section.

Why is semen analysis important?

Semen analysis provides insights to the reproductive health of the male. Laboratory examination of semen is used for the assessment of:

1. Male reproductive function;
2. Fertility potential of the male and may assist in choosing the appropriate treatment;
3. Efficacy of male contraceptive methods; and
4. Exposure to environmental pollution, drug, irradiation, or other toxic chemicals on reproduction in animals and men in epidemiological studies.

How is semen analyzed?

The World Health Organization developed a laboratory manual to provide a guide regarding acceptable methods for semen analysis in man. The goal of this manual is to contribute to improvement of assessment of male reproductive function and to standardize semen analyses so that results are comparable throughout the world. Most of the techniques can be applied to other species. In rodents, the

ejaculated semen forms a coagulum and does not liquefy, thus sperm parameters are assessed by examining spermatozoa in the cauda (tail region) of the epididymis. In rabbits, semen can by collected by an artificial vagina. In other species such, as cattle, horse, and many of the zoo animals, semen is collected by electro- ejaculation. In monkeys, stimulation using a penile cuff appears to be more efficient than electro-ejaculation. In men, semen is obtained for analyses by masturbation. Collection using a condom during intercourse is not recommended because of the presence of spermicidal or interfering agents in some condoms and loss of part of the ejaculate during intercourse. The duration of abstinence is noted because short periods of abstinence are associated with low semen volumes and sperm numbers. The sample is allowed to liquefy (by proteases present in the seminal fluid) and the basic parameters that are usually assessed are:

- volume, viscosity, appearance, and pH of the seminal fluid;
- sperm aggregation and presence of other cells (light microscopy);
- sperm motility using light microscopy;
- sperm concentration using a hemocytometer (counting chamber) under the microscope;
- sperm vitality after staining;
- sperm morphology after preparation of smear on a slide;
- other special tests as indicated.

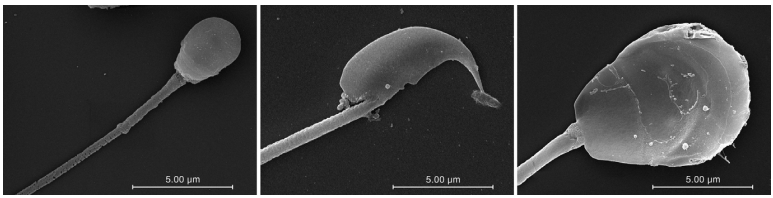

Figure 2. Scanning electron micrographs of human (left), mouse (middle), and guinea pig (right). Spermatozoa. Courtesy of G. Hunnicutt, The Population Council

What is a "normal" semen analysis in fertile man?

There are fluctuations in semen parameters from day to day in men and usually two semen samples are required to diagnose that the semen quality is below the distribution limits of fertile men. The World Health Organization in 2010 determined distribution of semen parameter from fertile men with a time to pregnancy of < 12 months and used the 5th percentile as the lower distribution limits. A follow-on study in 2021 included data from the 2010 study and added new data that resulted 3589 fertile men (with a time to pregnancy of < 12 month, defined period of abstinence between 2 to 7 days,) living in five continents to provide distribution limits of semen and sperm variables of adult men. In the latest edition of the World Health Organization laboratory manual, the lower 5th percentile for semen parameters in "fertile" adult men are:

- semen volume 1.4 mL;
- sperm concentration 16 million/ml;
- total sperm number per ejaculate 39 million;
- sperm progressive motility 30%, total sperm motility 42%;
- sperm vitality 54 % alive;
- spermatozoa with normal morphology 4 % (using strict criteria that exclude any spermatozoa with even a mild abnormality).

It is important to note that there is substantial overlap of sperm parameters from fertile and infertile men. These distribution limits are from fertile men, values above or below the 5th percentile do not provide a clear boundary between fertility and infertility.

What other tests on semen/sperm may be useful to assess male reproductive function?

The ability of the live sperm tail to swell under hypo-osmotic conditions is a test of sperm membrane integrity and sperm vitality. Special staining may reveal white blood cells in semen samples; this is suggestive of an infection. Semen biochemistry is indicated when accessory organs problems are suspected, e.g., semen fructose is low in men with congenital absence of the vas deference and seminal vesicles.

Other tests to assess sperm quality may be useful for diagnosis of fertile and infertile men and for research purposes but not recommended for routine analyses until clear predictive values are defined. Multiparametric sperm morphology assessment compiles data obtained at routine analyses into an index that may provide

increased accuracy and consistency of sperm morphology. Acrosome reaction, which occurs near the oocyte in the fallopian tubules, is a process where the acrosomal membrane fuses to expose the sperm head for fertilization of the oocyte (Chapter 25). The percentage of induced acrosome reacted sperm has been associated with fertilization rate. Sperm DNA quality and fragmentation are associated with miscarriage rates and in vitro fertilization outcomes and are used is studies on exposure to testicular toxicants (Chapter 23 and 42). The use of DNA fragmentation in assessment of male infertility is controversial. Computer aided assessment of sperm motility characteristics are not very useful for clinical diagnosis but is frequently used in research and epidemiological studies. The ability of sperm to exhibit hyperactivated motility is essential for sperm to migrate through the female reproductive tract and penetrate the zona pellucida. Computer aided sperm analyses is useful in the characterization of "hyperactivated motility" and may provide some insight on the signaling systems necessary for fertilization (Chapter 25). New developments allow men to check the presence of motile sperm by using apps on a mobile phone, but current methods lack accuracy when compared to laboratory-based methods. There are also at-home use kits for estimating the sperm concentration using immunoprecipitation. Evolving technology include use of genomic and epigenomic testing of sperm to define the causes of abnormal sperm head and flagella.

How does semen analysis help in assessment of male reproductive disorders?

Semen analysis is the cornerstone of the assessment of the reproductive capacity of male animals. Rodent semen does not liquefy and cannot be used for analyses. For animal breeders, semen analyses provide a guide to determine which animal should serve as a sire for multiple generations of animals. Semen analyses are used by reproductive toxicologists to study effect of exposure to environmental factors and toxicants on male reproductive function (Chapters 41, 42). Suppression of the number, motility, or function of spermatozoa in the ejaculate to very low levels is the goal of male contraceptive development (Chapter 30, 31). However, in clinical medicine semen analyses is used primarily for the diagnosis and treatment of male infertility (Chapters 33, 35). The diagnosis for male infertility is dependent, in large part, on the analysis of semen samples. Most men diagnosed with infertility have defects in spermatogenesis resulting

in low sperm concentration (oligozoospermia), which is generally associated with poor sperm motility (asthenozoospermia) and increased abnormal sperm morphology (teratozoospermia). Thus, when semen analyses show grossly low values, male factor infertility is diagnosed. The concentration of spermatozoa and their quality guide the clinician to determine the appropriate treatment and estimation of the prognosis.

How useful is semen analyses in predicting fertility potential in men? This was examined in a prospective study relating semen quality to probability of conception in 430 couples with first-time pregnancies. This study showed that increasing sperm concentration up to 40 million/mL were associated with increasing conception probability. The proportion of sperm with normal morphology was strongly related with likelihood of pregnancy. In another study sperm concentration, total progressive motility, normal morphology, and hypoosmotic swelling test (detects viable sperm) correctly identify 84.1% of 111 fertile men and 88.1% of 109 infertile men. In association studies, time to pregnancy is best predicted by combination of sperm concentration, motility, and morphology. Thus, from these prospective clinical studies, sperm concentration, total sperm count, sperm progressive motility, and the proportion of sperm with normal morphology are important predictors of male fertility up to certain thresholds. Increasing these parameters to beyond these thresholds did not appear to increase the probability of conception. How useful are additional functional tests in predicting fertility has not been studied in prospective large scale clinical studies.

Suggested reading

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