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

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What composes semen?

Semen consists of spermatozoa mixed with secretions from the testis (Chapter 6) and epididymis (Chapter 10) which during ejaculation are diluted with secretions from the prostate gland (Chapter 11) and seminal vesicles. The structure of the human spermatozoon is shown in Fig.1. The spermatozoon consists of the head which has the nucleus (chromatin containing the genetic material) covered by the acrosome; the mid-piece with the mitochondrion; and the tail (flagellum or principal piece) with microtubules forming the axoneme enclosed by the fibrous sheath which 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.

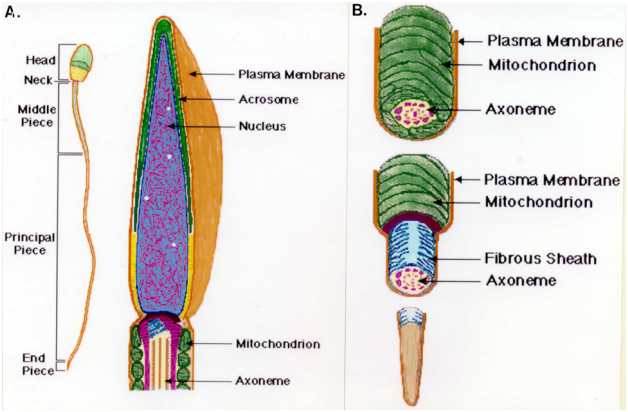

FIG. 1. A. Major elements of a human spermatozoon that are common to mammalian species. B. Middle piece (top), principal piece (middle), and end piece (bottom) of a spermatozoon viewed in cross-section.

The seminal fluid is made up a mixture of secretions from the testis, epididymis, prostate and seminal vesicles and 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, there are some reports indicating that semen samples collected by masturbation may be of a

lower quality than those recovered during sexual intercourse. Because the volume of the seminal fluid may be quite variable, it has been suggested that the total number of spermatozoa in the ejaculate may be a more important parameter than the sperm concentration in the ejaculate.

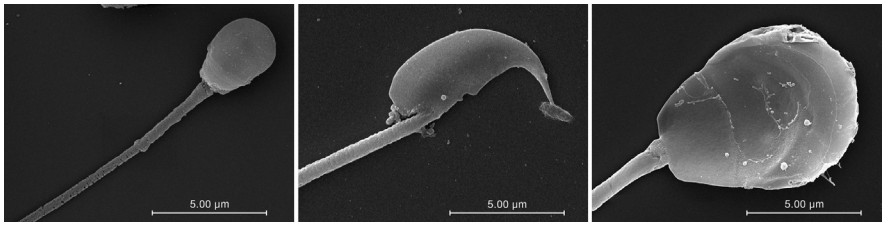

FIG. 2. Scanning electron micrographs of human (left), mouse (middle), and guinea pig (right). Spermatozoa. Courtesy of G. Hunnicutt, The Population Council.

How is semen analyzed?

The World Health Organization (WHO, 2009) has developed a manual to provide a guide regarding acceptable approaches to semen analysis in man. This section will focus on these norms. Most of the techniques can be applied to other species. In rodents, the ejaculated semen forms a coagulum and does not liquefy, thus sperm counts are done by examining the 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 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 following are usually assessed:

- 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.

There are fluctuations in semen parameters from day to day in men and usually at least two semen samples are requried to diagnose that the semen quality is below the reference range of adult men. A recent study of over 4500 samples from men living in four continents gives the reference range of adult men. The lower reference limits (5^{th} percentile) for semen parameters in "fertile" adult men are:

- semen volume 1.5 mL
- total sperm number per ejaculate 39 million
- sperm concentration 15 million/mL
- sperm progressive motility 32%, total sperm motility 40%
- sperm vitality 58 % alive
- spermatozoa with normal morphology (using strict criteria that exclude any spermatozoa with even a mild abnormality) 3 %.

Other tests to assess sperm function may include the ability of spermatozoa to interact and penetrate human cervical mucus and sperm antibody tests when sperm aggregates are noted. Special staining may reveal white blood cells in semen samples; this is suggestive of an infection. Semen biochemistry is indicated only when accessory organs problems are suspected, e.g., semen fructose is low in men with congenital absence of the vas deference and seminal vesicles. Assessment of sperm function may include tests of sperm chromatin (sperm damage, Chapter 13); the ability of the sperm to swell under hypo-osmotic conditions (test of sperm membrane integrity); the ability of the sperm head to lose the acrosome cap upon stimulation (acrosome reaction, a pre-requisite for fertilization); whether spermatozoa can bind human zona pellucida (testing the ability of spermatozoa to bind to zona) and penetrate a zona free hamster egg (the ability to penetrate the egg membrane and fertilize an oocyte). Computer assisted assessment of sperm motility characteristics has not proven to be very useful for clinical diagnosis but is frequently used in research and epidemiological studies.

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

Semen analysis is usually 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

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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 environment and toxicants on male reproductive function. Suppression of the number or motility of spermatozoa in the ejaculate to very low levels is the goal of male contraceptive development. However, in clinical medicine semen analyses is used primarily for the diagnosis and treatment of male infertility. The diagnosis for male infertility is dependent, in large part, on the analysis of semen samples. Most men diagnosed with 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 showed grossly low values male factor infertility is diagnosed. The concentration and the quality of sperm parameters 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, the time to pregnancy in 942 couples increased with sperm concentration up to 55 million/mL and percent spermatozoa with normal morphology to 19 %. Thus from these prospective large clinical studies, sperm concentration, total sperm count 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 conception probability. How useful are additional functional tests in predicting fertility has not been studied in prospective large scale clinical studies.

Suggested reading

Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, Henriksen TB, Scheike T, Giwercman A, Olsen J, Skakkebaek NE Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet. 1998; 352: 1172-77.

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