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Semen analysis : a review

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A semen analysis is the examination of freshly ejaculated seminal fluid. Seminal fluid is a viscous, turbid fluid produced mainly from secretions of the seminal vesicles (45–80% of volume) and prostate gland (15–30% of the volume). About 1% of the total volume is spermatozoa and testicular fluid produced by the testes. A routine analysis of seminal fluid includes the measurement of fluid volume, viscosity, pH, and fructose and measurement of sperm concentration, count, motility, viability, and morphology. Additional tests are performed as indicated. These are usually performed by andrology laboratories and include testing for sperm auto antibodies, zona free hamster oocyte penetration, cervical mucus penetration, the acrosomal reaction test, and computer assisted sperm analysis (CASA).

A semen analysis is the examination of a male's ejaculate, performed to determine if the cause of a couple's infertility is attributed to the male's inability to fertilize the ovum. It is also used to confirm the absence of sperm following vasectomy. The patient should abstain from intercourse for three days prior sample collection and refrain from drinking alcoholic beverages for at least 24 hours before testing. Additionally, several herbal supplements have been found to affect sperm counts and/or characteristics.

A semen specimen to investigate infertility must kept at room temperature. It should be collected by masturbation into a disposable sterile, wide-mouth container. A room close to the testing site is preferred for collection, since specimen quality deteriorates rapidly. If possible, examinations for motility and viability should be performed and smears prepared within one hour of collection. Timing is not as critical for post vasectomy testing. Physiological and environmental factors can increase the variability of semen analysis, and the World Health Organization (WHO) recommends the evaluation of two ejaculates collected at least seven days but not more than three months apart.

Physical characteristics of the semen sample that are evaluated include volume, gross appearance (color, turbidity), viscosity, and liquifaction. Seminal fluid willcoagulate within five minutes of collection due to coagulating protein secreted by seminal vesicles. The seminal fluid should liquefy within one hour at room temperature, due to the action of prostatic secretions. Failure to do so inhibits motility. After liquifaction, viscosity may be measured by observing the fluid as it drains from the tip of a 5 mL serological pipet. The fluid should flow from the tip in discrete droplets. Formation of a thread of two or more centimeters at the tip indicates abnormally high viscosity. Volume is determined by determining the amount of fluid that can be drawn into a 10 mL serological pipet.

Male infertility may be caused by many conditions that affect the production of functional sperm. The most common cause is varicocele (hardening of the veins that drain the testes) which accounts for about 40% of cases and is treated surgically. Testicular accounts for approximately 10% of cases and may result from numerous causes including malignancy, mumps, Kleinfelter's syndrome, injury, and radio- or chemotherapy. Hyperspermia, increased seminal fluid volume, also accounts for about 10% of cases. Endocrine diseases affecting spermatogenesis account for approximately 9% of cases and usually

involve pituitary or adrenal hypoplasia or hyperthyroidism. Obstruction of the ejaculatory duct accounts for about 5% of cases and sperm auto antibodies for 1–2%.

Autoantibody: An antibody formed in response to, and reacting against, an antigenicconstituent of the individual's own tissues.

Infertility: The diminution of absence of ability to produce offspring.

Morphology: The biological study of the form and function of living organisms.

Motility: The the ability to move spontaneously.

Semen: Fluid discharged at ejaculation in the male, consisting of secretion of glands associated with the urogenital tract and containing spermatozoa.

Sperm: Vernacular term for spermatozoon, which is a mature male germ cell, the specific output of the testes, which impregnates the ovum in sexual reproduction.

Vasectomy: An operation done to sterilize a man by stopping the release of sperm into semen.

Sperm counting methods

The sperm concentration is usually performed using a 1:20 dilution of seminal fluid in a diluent containing formalin which immobilizes the sperm. Usually five of the 0.2 x 0.2 mm squares of a hemacytometer grid are counted. The number of cells counted is equal to the sperm concentration in millions per mL. All 25 squares are counted if there are less than 10 sperm (spermatozoons) per square. A Mackler chamber, a grid consisting of 1 square millimeter divided into 100 equal squares, (0.1 x 0.1 mm, 0.01 mm deep) can be used in place of a hemacytometer. Undiluted seminal fluid is heated to 50-60°C to immobilize the sperm. Heads are counted in 10 of the squares and the total is equal to the sperm concentration in millions per milliliter. A sperm concentration less than 20 million per milliliter is termed oligozoospermia, and often results from ductal obstruction, regurgitation of sperm into bladder, or testicular failure. The total sperm count is determined by multiplying the sperm concentration per mL by the seminal fluid volume.

Motility and viability evaluation

Sperm motility should be performed within one hour of ejaculation. During this portion of the analysis, 10 microliters of semen are placed on a standard microscopic slide, and a coverglass is applied. Ten random fields are thenexamined at 250x to 400x magnification using phase contrast or brightfield microscopy. Two hundred sperm are graded for motility using a scale ranging from zero to four, with zero signifying no motility, and four describing sperm that exhibit rapid, progressive movement. A more detailed analysis of sperm motility is obtained using CASA. This procedure produces a computer analysis of video camera generated microscopic images of sperm movements. Various aspects of sperm movement such as curvilinear and straight-line velocity are measured and analyzed statistically.

Sperm viability is measured when the motility test is abnormal. The test is based upon the fact that living sperm exclude the dye eosin Y, but dead sperm do not. A slide is prepared by mixing a sample of the seminal fluid and the dye, and 200 sperm are counted under the microscope. The percentage of living sperm (unstained sperm) is recorded.

Morphology evaluation

The morphology of sperm is also assessed from a stained smear. To prepare a stained specimen, a drop of semen is placed on a glass slide and a second slide is used to spread the drop over the slide surface. The smear is air dried and fixed using ethanol-ether. The slide is stained. Two hundred mature sperm are evaluated for head, neck, and tail defects. Any white blood cells (WBCs) or immature sperm cells (round cells) are also counted per 200 mature sperm. WBCs and rounds cells are estimated from the average number seen per 400x field. When more than one WBC or five round cells are seen on average per field, the respective cell count should be performed. Each cell per field equates to approximately one million cells per mL of seminal fluid. There are two alternative ways of classifying sperm that give very different results. The majority of clinical labs use nonstrict criteria. This process identifies only gross abnormalities of the sperm. The alternative method, strict criteria, is used by andrology and some clinical labs. For example, the WHO criteria for the normal sperm head is as follows: length 4.0–4.5 microns, width 2.5–3.5 microns, length:width ratio 1.5–1.75, acrosomal area 40–70%, vacuolization less than 20% of head area. Studies have shown that the probability of successful fertilization via assisted reproductive technology diminishes significantly when less than 15% of the sperm are normal by strict criteria. There are several strict criteria in use, including those developed by WHO. Chemical tests routinely performed on seminal fluid include pH and fructose. pH is measured with pH paper, and fructose may be measured quantitatively using an enzymatic assay.

Antibodies to sperm have the potential to impair fertility. While sperm agglutinins (antibodies) may be detected in the male or female partner of up to 10% of infertile couples, they are not always responsible for infertility. There are several tests for sperm agglutinins including direct microscopic observation, the mixed antiglobulin test, and the immunobead test.

Collection process (how to collect specimen for analysis)

Sterile, wide-mouth containers should be used for specimen collection. The best quality sperm are obtained when the specimen is collected after three days of sexual abstinence, but not more than five to seven days. Because the initial portion of the ejaculate contains the majority of the sperm cells, and the volume of ejaculate may provide clues regarding infertility, coitus interruptus should not be used as the method of collection.

The specimen should be examined in the laboratory within one hour of collection. Typically, two to three specimens are examined over a period of several weeks. When results from two testing days are different, additional specimens collected over a two- to three-month period should be analyzed. Each laboratory defines its own set of normal values but the recommendation of WHO are valuable. Here are some normal values of sperm analysis.

- Volume: 2.0–5.0 mL.
- pH: 7.2–8.0.
- Sperm concentration: greater than or equal to 20 million per mL.
- Sperm count: greater than or equal to 40 million per ejaculate.
- Motility: Greater than or equal to 50% demonstrating forward progressive movement or 25% or more demonstrating rapid progressive movement. Prolonged abstinence may depress sperm motility. Frequent sperm agglutination suggests the presence of antisperm antibodies.
- Morphology: Nonstrict criteria: greater than 60% normal sperm. Strict criteria: 15–40% normal sperm.
- White blood cells: less than 1 million per mL.
- Round cells: less than 5 million per mL.
- Viability: equal to or greater than 75%.

- Viscosity: droplets to threads less than 2 cm.

Post-vasectomy semen analysis

Semen analysis to confirm the success of a vasectomy is concerned only with the absence or presence of sperm. Semen is collected six weeks after surgery, or after at least 20 ejaculations. Sperm should not be seen. If sperm are seen, another specimen is collected two to four weeks later. The test should be repeated several months later to ensure that the vas deferens have not reattached.

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