Microscopic Examination of Seminal Fluid: A review

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Absract

Microscopic examination includes the determination of sperm motility, concentration, morphology, and viability; the concentration of other cells present; and the presence of sperm agglutination. Some laboratories use a single stain for the evaluation of several parameters, such as eosinnigrosin stain for sperm vitality, morphology, and the identification of other cells, whereas others use different stains that specifically enhance each parameter to aid in the identification and evaluation of sperm and other cells.

Keywords: sperm, normal, morphology, viability

1. Motility

Motility is one of the most important characteristics of sperm because immotile sperm, even in high concentrations, are unable to reach and fertilize an ovum.

Sperm motility is evaluated subjectively and semiquantitatively using phasecontrast microscopy (brightfield microscopy can also be used with appropriate condenser adjustments). After complete liquefaction, the semen sample is mixed well to ensure homogeneity. A consistent volume of each specimen is evaluated by pipetting a fixed volume (e.g., 10 or 20 µL) of semen onto a microscope slide using a calibrated positive-displacement pipette. The sample is covered with a coverslip of predetermined size (e.g., 18 x 18 mm), and the slide is allowed it to settle for about 1 minute before evaluation. To enhance the accuracy and precision of results, wet mounts of each sample should be prepared and evaluated in duplicate. Because sperm motility is affected adversely by temperature, some laboratories control the temperature of the microscope slide at 37°C using an air curtain incubator. Others perform the analysis at room temperature.

(Table: 1) Sperm Motility Grading Criteria	
0	Immotile
1	Motile, without forward progression
2	Motile, with slow nonlinear or meandering progression
3	Motile, with moderate linear (forward) progression
4	Motile, with strong linear (forward) progression

Initially, each wet mount is screened to ensure uniformity in sperm movement throughout the preparation. Next, sperm motility is graded subjectively from 0 to 4 under 200 x (or 400 x) magnification. Table: 1 show typical grading criteria used to evaluate sperm motility. Some laboratories use a manual cell counter and evaluate the motility characteristics in 100 sperm, whereas others grade the sperm encountered in 6 to 10 high-power fields (400 x).

The speed and forward progression of each sperm are evaluated. In normal semen evaluated within 60 minutes of collection, 50% or more of the sperm will show moderate to strong linear or forward progression.

Concentration and Sperm Count

For fertility purposes, the actual number of sperm is not as important as other characteristics. The concentration of sperm in an ejaculate is considered normal when 20 to 250 million per milliliter of sperm are present; values less than or greater than this range are considered abnormal and are associated with infertility. The variation in the sperm concentration within a single individual can be significant and depends partially on the period of sexual abstinence but can also be affected by viral infection and stress. For these reasons, multiple semen specimens should be evaluated to reliably assess the quantity and quality of an individual's sperm.

Manually, the concentration of sperm is determined by using a hemacytometer after preparing an appropriate dilution of the semen. Frequently, a 1 : 20 dilution is prepared. If during initial microscopic examination, the sperm concentration is noted to be exceptionally high or low, a new dilution can be prepared and mounted. All dilutions should be made using a calibrated positive displacement pipette to deliver the semen quantitatively to a premeasured amount of diluent. After the hemacytometer is filled with the well-mixed dilution of semen, it is placed in a humidifying chamber and allowed to settle for 3 to 5 minutes before counting. The type of hemacytometer, the specimen dilution used, and the areas counted determine the conversion factor necessary to obtain the concentration of sperm in millions per milliliter.

Several alternative manual counting methods have been developed, such as the Makler chamber (MidAtlantic Diagnostics, Mt Laurel, NJ), Horwell, Cell VU chambers (Millennium Sciences, NY), Microcell slides (Conception Technologies, San

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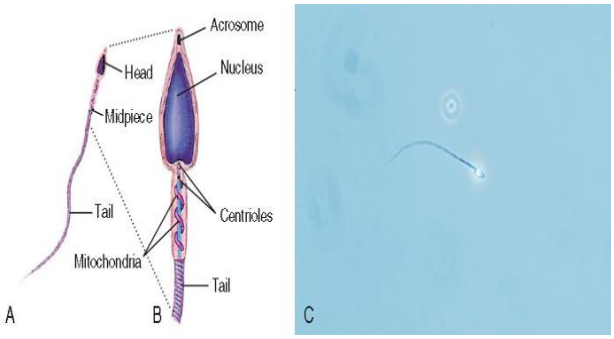
Diego, CA), and Leja slides (Leja, The Netherlands). Studies vary in their outcomes—some supporting the manual hemacytometer method as the method of choice for sperm counting, other studies found better accuracy and precision using an alternative counting chamber. Regardless of the method used, the dilution of the semen is always a potential source for error and requires the utmost attention to ensure accurate and reproducible technique. The counting of motile sperm and high sperm concentrations has also been identified as two sources of error. In contrast to sperm concentration (sperm per milliliter), the sperm count is the total number of sperm present in the entire ejaculate. This value is calculated by multiplying the sperm concentration (sperm/mL) by the total volume of the ejaculate.

Sperm count = Sperm concentration (sperm/mL) × Volume of ejaculate (mL)

Morphology

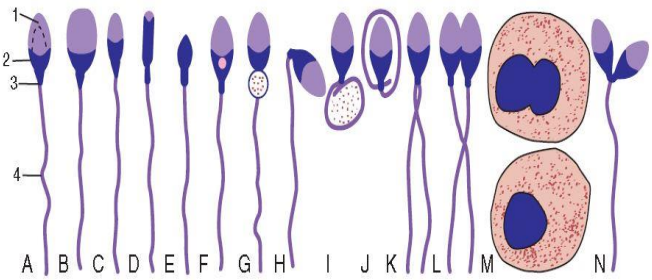
Sperm morphometry—measurement of the sperm head length, width, circumference, and area—enables the generation of objective data. To be considered normal, sperm must meet strict criteria regarding their size and shape, which can be determined by computerized systems or manually using a microscope with a calibrated ocular micrometer.

Human sperm have three distinct areas: head, mid piece, and tail. When viewed from the side, sperm appear to be arrowhead shaped (Figure 1). When viewed from the top, normal human sperm have oval heads that are 2.5 to 3.5 µm in width and 4.0 to 5.0 µm in length. Only sperm lying flat should be evaluated and their head length-to-width ratio should be 1.50 to 1.75. Spermatozoa with values outside these ranges are considered abnormal.



(Figure: 1) Sperm. A. A schematic of a mature sperm. B. An enlarged view of head and midpiece. C. A photomicrograph of a single sperm using phase-contrast microscopy, (400x)

The mid piece, located immediately behind the head, is 6 to 7.5 µm long and is thicker than the tail, but not greater than 1 µm in width. The tail should be slender, uncoiled, and at least 45 µm long. When a "basic" morphology evaluation is performed, each spermatozoon (single sperm cell) is identified simply as normal or abnormal with the percent of normal forms reported. If a "complete" morphology evaluation is performed, then each spermatozoon is classified using five categories: normal, head defects, midpiece defects, tail defects, and cytoplasmic droplet present. Cytoplasmic droplets are usually located in the midpiece region and are considered abnormal if this region is greater than one-third the area of a normal sperm head. The head can contain vacuoles, but they are not considered abnormal unless they occupy more than 20% of the head. Note that a single spermatozoon can have multiple defects, and each defect is documented. (Figure: 2) depicts a normal spermatozoon and a variety of abnormal forms.



(Figure: 2) Sperm morphology. A. Normal spermatozoon: 1, acrosome; 2, postacrosomal cap; 3, midpiece; 4, tail. B. Large head. C. Tapered head. D. Tapered head with acrosome deficiency. E. Acrosomal deficiency. F. Head vacuole. G. Midpiece defect—cytoplasmic extrusion mass. H. Bent tail. I and J. Coiled tails. K. Double tail. L. Pairing phenomenon. M. Sperm precursors (spermatids). N. Double-headed (bicephalic) sperm.

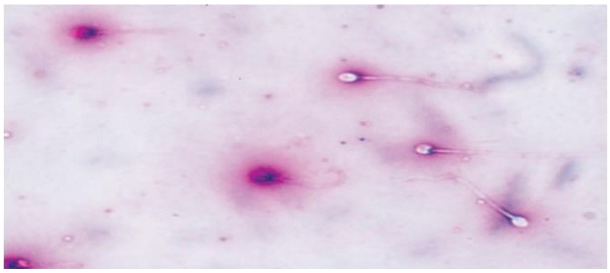
To manually evaluate sperm morphology, smears of fresh semen are made, air dried, and stained. The smears can be made similar to those for traditional blood smears by placing a drop (10 to 15 µL) of semen near one end of a clean microscope slide. Using the edge of another slide, the drop is allowed to spread along the edge of the second slide, and then the edge of the second slide is moved forward, dragging the semen sample across the surface of the first slide and producing a smear. An alternate technique involves placing the second slide over the first and allowing the semen to spread between them. Once spreading is complete, the slides are pulled apart and allowed to air dry. Staining enhances the visualization of sperm morphology and enables the identification and differentiation of white blood cells, epithelial cells of the urethra, and immature spermatogenic cells (i.e., spermatids, spermatocytes, and spermatogonia). Giemsa, Wright's, and Papanicolaou stains are frequently used. These stains differ with respect to complexity and turnaround time.

Using oil immersion (1000 x) and an area of the slide where sperm are evenly distributed, 200 sperm are classified. Note that morphologically abnormal sperm are found in all semen specimens. Abnormalities may involve all or only one region of the spermatozoon and can affect its size, shape, or both. In addition, numerous sperm variations are found within a single ejaculate. Although some morphologic abnormalities have been associated with particular disorders (e.g., tapered heads with

varicocele), most abnormalities are nonspecific.

Vitality

Vital staining of a fresh semen smear enables rapid differentiation of live and dead sperm. Because dead sperm have damaged plasma membranes, these cells take up stain; living sperm do not (Figure: 3). When a large percentage of immotile sperm are observed, this evaluation determines whether the sperm are immotile because they are dead or because of a structural abnormality (e.g., defective flagellum).



(Figure: 3) Sperm vitality using eosin-nigrosin (Bloom's) stain. White sperm were alive; pink-stained sperm were dead. Brightfield microscopy (400x).

Eosin alone or an eosin-nigrosin (a modification of Blom's technique) combination is frequently used to determine sperm vitality. Using brightfield or phasecontrast microscopy and 1000 x (or 400 x), 100 sperm on a stained smear are evaluated. The percentage of dead sperm cells should not exceed the percentage of immotile sperm. In other words, if 65% of the sperm in a semen specimen are dead, the motility cannot exceed 35%. Hence the vitality evaluation provides a convenient quality or cross-check of the motility evaluation. In fresh normal semen, 50% or more of the sperm are alive.

2. Agglutination

Agglutination, the sticking together of motile sperm, is evident by microscopic examination of a wet preparation. Although some clumping of immotile sperm may occur in normal semen specimens, the observation of distinct head-to-head, head-to-tail, or tail-to-tail orientation of sperm is associated with the presence of sperm-agglutinating antibodies. The extent of true agglutination is often graded as "few," "moderate", or "many". Even a small amount of true agglutination is significant and indicates the need for further evaluation.

Immunoglobulin G and immunoglobulin A antibodies bound to sperm have been identified and correlated with reduced fertility. This is known as immunologic infertility.

Macroscopic and microscopic tests are available to detect and determine the immunoglobulin class of sperm antibodies (IgG, IgA). Both tests produce comparable results, but the mixed agglutination reaction (MAR) test is rapid (≈3 minutes) and easy to perform, whereas the immunobead test is time-consuming (≈45 minutes), technically more complicated, and more expensive. The cutoff values for these tests vary between laboratories.

3. Conclusion

A semen analysis is used to find out if a problem with semen or sperm may be causing a man's infertility. The test may also be used to see if a vasectomy has been successful. A vasectomy is a surgical procedure that is used to prevent pregnancy by blocking the release of sperm during sex

4. References

Marconi M, Nowotny A, Pantke P, (2008). Antisperm antibodies detected by mixed agglutination reaction and immunobead test are not associated with chronic inflammation and infection of the seminal tract. Andrologia 40:227,

Jeyendran RS: Sperm collection and processing: a practical guide, New York, 2003, Cambridge University Press.

World Health Organization: WHO laboratory manual for the examination and processing of human semen, ed 5, Geneva, Switzerland, 2010, World Health Organization.

Tomlinson M, Turner J, Powell G. (2001) One step disposable chambers for sperm concentration and motility assessments: how do they compare with the World Health Organization's recommended methods? Hum Reprod 16:121, 2001.

Amelar RD, Dubin L: Semen analysis. In Amelar RD, Dubin L, Walsh PC, editors: Male infertility, Philadelphia, 1977, WB Saunders.

Eliasson R. (2010) Semen analysis with regard to sperm number, sperm morphology and functional aspects. Asian J Androl; 12:26–32

Keel BA. (1990) The semen analysis. In: Keel B, Webster B, editors. CRC Handbook of the laboratory diagnosis and treatment of infertility. Boca Raton: CRC Press; 1990. pp. 27–69

Björndahl L. (2010). The usefulness and significance of assessing rapidly progressive spermatozoa. Asian J Androl.; 12:33–5.

Van Waart J, Kruger TF, Lombard CJ, Ombelet W. (2001) Predictive value of normal sperm morphology in intrauterine insemination (IUI): a structured literature review. Hum Reprod Update; 7:495–500

Punab M, Lõivukene K, Kermes K, Mändar R. (2001). The limit of leucocytospermia from the microbiological viewpoint. Andrologia; 35:27–8.

Quintero I, Ghersevich S, Caille A, Munuce MJ, Daniele SM, Morisoli L. (2005). Effects of human oviductal in vitro secretion on spermatozoa and search of sperm-oviductal proteins interactions. Int J Androl.;28:137–43