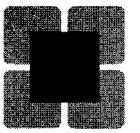

Laboratory diagnosis in andrology

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■ Evaluation of male fertility traditionally has relied on analysis of spermatozoa and seminal plasma. Technology has consisted almost exclusively of subjective motility assessment and morphology of stained sperm-cell preparations. Evaluation of seminal plasma components, particularly anti-sperm antibodies, was based on functional properties of sperm immobilization and agglutination. In the last few years, objective measurements of sperm motility have become available, and new methods for immunological evaluation have been applied to andrology laboratory evaluations. This review discusses methods of laboratory diagnosis of male infertility, including many of these new technologies.

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ANDROLOGY, the study of male reproductive function, is a relatively new clinical and laboratory specialty. The recognition that the male could have defective or suboptimal reproductive capacity that contributed to a couple's infertility has been acknowledged only within the past 40 years.[1] Gradually, this idea has been accepted, and responsible fertility specialists consider evaluation of the male partner a critical component of a comprehensive fertility examination. Male fertility can be expressed only through a female partner; thus, true fertility is difficult to evaluate because it depends critically on the reproductive health of the woman.[2] Because the field is relatively young, development of diagnostic and therapeutic studies is limited. In the last few years, new technologies have become available that should improve ability to diagnose male infertility.

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SPECIMEN COLLECTION

A semen specimen for analysis is usually obtained by masturbation. Some evidence suggests that the degree of erotic stimulation directly affects the quality of the specimen. One report has shown that the quality of semen obtained by intercourse with collection into a Silastic condom is superior to ejaculates produced by masturbation.[3] The use of this condom also permits collection of semen from patients with religious proscriptions forbidding masturbation. In any case, obtaining the sample requires that the ordering physician and the laboratory personnel understand the embarrassment the patient often feels and take measures to reduce his anxiety.

The timing of analysis is critical to proper interpretation of results. Specimens collected within 24 hours of any previous ejaculation or longer than five to seven days later may not represent the optimal count or motility due to depletion or aging in the ejaculatory ducts.[4] Moreover, at least three semen analyses should be performed, each one to two weeks apart, to establish a patient's usual semen quality.[5] Specimens should be received by the laboratory within one hour of collection. On-site collection is preferable as it permits observation

FIGURE 1. Computerized semen analysis instrument.

of the sample immediately after ejaculation and through the liquefaction stages. Sperm motility decreases approximately 5% per hour in ejaculates from normal men and may decrease more rapidly in men with sperm abnormalities; thus, examination of specimens after three hours can sometimes be useful in identifying "subfertile" men.[6]

ENUMERATION AND MORPHOLOGY OF SPERMATOZOA

Semen analysis has progressed a long way since the 17th century when Leeuwenhoek first identified spermatozoa, which he called "animalcules."[7] He and other scientists of his day were certain that they could see the shape of a person, or homunculus, inside the sperm head. Analyses using modern miscroscopes have failed to confirm this observation.

Most investigators have used permanent, Papanicolaou-stained smears to evaluate morphology, which should reveal at least 50% normal forms.[8] New computer-integrated digitizers can perform morphometric analyses of sperm. Although few correlations with fertility have been published, early reports indicate that the sperm-head length-to-width ratio appears to be decreased in infertile men.[9] Electron microscopy can be used to determine the precise nature of submicroscopic morphological defects and is increasingly valuable in diagnosing tail defects that result in impaired motility.[10]

The normal range for sperm density is quite wide. Most authors have avoided stating an upper end of the normal range and instead have attempted to define minimum standards of adequacy. A series of studies in the early 1950s by MacLeod and Gold[11] used sperm count, motility, morphology, and fertility status to define the boundaries of reduced male fertility, with 20 million sperm/mL of semen as the lower limit of normal. As clinical interpretation of these characteristics improved, other authors found that that original limit was too high. Bostofte et al[12] reviewed conception data in 1,639 couples and showed that about 25% of wives of men with counts lower than 5 million/mL became pregnant and about 50% of wives of men with counts between 5 and 20 million/mL became pregnant. However, low counts and other adverse sperm and semen parameters often are related; thus the boundary delineated by MacLeod and Gold serves to alert the clinician to look for other abnormalities.

MOTILITY ASSESSMENT

The development of a specialized chamber by Makler provided an opportunity to count live sperm. The Makler chamber is a precisely machined, gridded chamber with a depth of 10 μm that permits free horizontal sperm motion in a single plane throughout the medium.[13] Normally, 60% of the sperm are motile[8]; a reading higher than 80% is rare in specimens where motility has not been artificially stimulated or enhanced in vitro.

Observation of live sperm using phase contrast permits best resolution. The addition of a video camera, recorder, and monitor adds modest expense and permits later review of archival tapes, which can be valuable in assessing the effectiveness of surgical or medical treatments.

In addition to motility, the progressiveness, or forward movement, of the sperm should be graded. Until recently, the assessment was subjectively graded using an arbitrary scale.[13] The sperm was examined using 20 high-power magnifications and was rated as follows:

0—no motion;

1—very slow tail beating, and no forward progress across the microscopic field;

2—slow forward progress present;

3—fast motion forward with the tail visible when stroking; and

4—very rapid movement with difficulty in visualization of tail movement.

Based on this scale, progressiveness should at least be 2.

A major problem with this assessment is that its summary nature does not distinguish different characteristics of sperm motion; thus specific defects are not readily identified. In addition, a great deal of interobserver and intraobserver variation is inherent in the estimate.

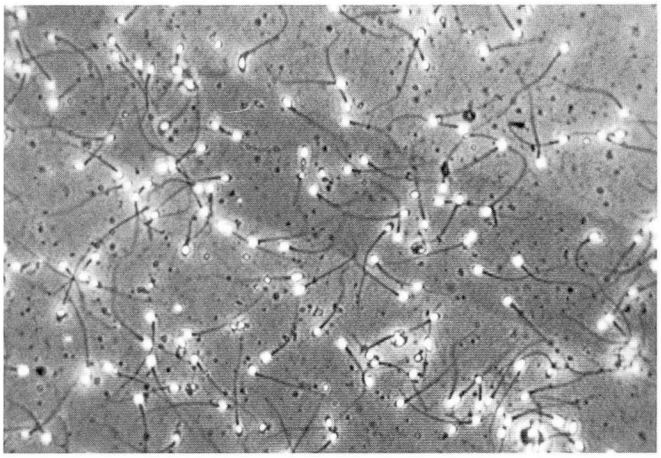

FIGURE 2. Video field of live sperm observed under phase contrast.

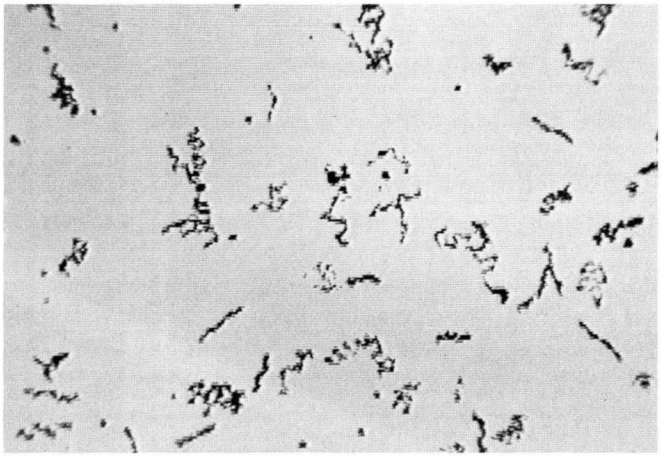

FIGURE 3. Digitized image of sperm.

Efforts to make these measurements less subjective and more accurate and specific first involved the use of multiple-exposure photography to determine actual sperm velocity.[15] Six or more exposures of moving sperm were superimposed to generate a "track" of sperm motion. By measuring the distance traveled during a given time, velocity could be computed. Obviously, this procedure was time consuming, so computer analysis of digitized images was subsequently developed. Nonetheless, the technician was required to photograph the sperm and trace the track with a digitizing apparatus, making this technique impractical for a busy laboratory. In the last three years, automated computer analysis of videomicrography-generated sperm images has been developed by several commercial sources,[8] making quantitative motion analysis readily available, albeit expensive (capital outlay, $20,000 to $40,000).

As shown in *Figure 1*, the components of an automated-computer-analysis system (Cell-Soft, CryoResources, New York, NY) include a phase-contrast microscope with high-intensity illumination, video camera and recorder, and two high-resolution monitors (one that shows the camera image and the other a digitized image). For examination, an undiluted semen specimen (5 μL) is placed onto the Makler chamber. The chamber is positioned on the microscope stage, and the image is transferred to a video monitor through the camera (*Figure 2*). The computer digitizes images of each sperm cell and calculates the path of trajectory. Each moving sperm forms a track as it moves; nonmotile sperm appear as dots. These tracks and dots appear on the screen of the second monitor as images are transferred from the first monitor (*Figure 3*). From these images, the computer counts the number of sperm and analyzes five sperm-motion parameters: percent motile, velocity, linearity, lateral head displacement amplitude, and beat/cross frequency (BCF).

Sperm-motion parameters are emerging as important indicators of male fertility. In a study by Holt et al,[16] sperm that penetrated a zona-free hamster egg had an average velocity of 34.2 ± 0.9 μm/sec, whereas sperm that was unable to penetrate had an average velocity of 24.2 ± 1.2 μm/sec. Milligan et al[17] observed that spermatozoa from fertile and longstanding infertile men could be distinguished on the basis of sperm velocity and suggested that 30 μm/sec was the lower limit of normal, a boundary that has been verified by Feneux et al,[18] Mathur et al,[8] Holt et al,[16] and our own observations.

Few investigations of lateral head displacement have been published, but this parameter appears to be an important indicator of sperm vigor. Feneux et al[18] reported that spermatozoa from fertile men had a lateral head displacement of 5.2 ± 0.4 μm; those from infertile men had a lateral head displacement of 1.6 ± 0.2 μm ($P<.05$). BCF also was lower in infertile men (4.8 ± 1.7 Hz) compared to fertile men (7.1 ± 1.8 Hz) ($P<.05$).

CERVICAL MUCUS PENETRATION

Cervical mucus filters out abnormal and poorly motile sperm while facilitating actively motile normal sperm; in addition, it provides a likely site for capacitation of the sperm.[19] Thus, the ability of the sperm to penetrate cervical mucus is an essential function and important aspect of a fertility work-up. Human cervical mucus, loaded into capillary tubes, can be used for test-

ing this interaction, but it is difficult to differentiate between the separate functional characteristics of the spermatozoa and mucus. However, bovine cervical mucus closely resembles midcycle human cervical mucus and has similar viscoelastic properties, ferning patterns, and glycoprotein composition.[20] Available evidence suggests that penetration of bovine cervical mucus is a good predictor of the ability of spermatozoa to penetrate human cervical mucus.[19,21] Flat capillary tubes, filled with bovine cervical mucus and stored frozen, are available commercially (Penetrak, Serono Diagnostics, Randolph, MA). For each analysis, a tube is thawed and incubated in a vertical position with a small aliquot of semen for 90 minutes at room temperature. The tube is placed on a calibrated slide, and the farthest distance traveled (in millimeters) by an individual sperm (the vanguard sperm) is recorded (Penetrak packet insert, Serono Diagnostics). Cervical mucus penetration by sperm from fertile men was significantly different from that of men with a history of infertility due to poor human cervical mucus penetration. Sixty-eight percent of the infertile men had penetration scores <20 mm, while 79% of the fertile men had scores >30 mm.[19]

ANTI-SPERM ANTIBODY DETERMINATION

The spontaneous occurrence of antibodies in sera and in male and female reproductive-tract fluids directed against spermatozoa has been well documented.[22–27] In laboratory animals, autoimmune aspermatogenesis and asthenospermia can be readily induced. However, the role of immune mechanisms in causing infertility is not well understood. In part, this can be attributed to relatively nonspecific and insensitive diagnostic tests used in the past. Evaluation of anti-sperm antibodies has relied on subjective observation of sperm agglutination and immobilization by serum, seminal plasma, or cervical mucus, with the assumption that these phenomena were due to the presence of antibody.[28] The most commonly used anti-sperm antibody screening test has been the gel agglutination test, as described by Kibrick et al,[29] which qualitatively measures macroscopic agglutination brought about by cross-linking of spermatozoa by multivalent antibodies. Another commonly used test has been the sperm motility-inhibition assay for complement-dependent cytotoxicity, as described by Isojima et al.[30] Both methods suffer from insensitivity and, moreover, a lack of specificity. Nonimmunoglobulin-mediated agglutination may yield false-positive results, and nonagglutinating antibodies may lead to false-negative results. Comparing results to actual immunoglobulin assays is not meaningful unless more than one antibody class is present; also, neither detects antibodies of the IgA class.[31]

In attempts to reduce these problems, methods have been formulated that detect actual immunoglobulin. Although enzyme-linked immunosorbant assays[32–34] and radioimmunoassays have been developed,[35] their reproducibility and correlation with fertility has been poor, probably due to the different techniques for preparing the test-sperm antigens.[36,37] The most reliable method uses polyacrylamide beads coated with rabbit anti-human immunoglobulin.[36–40] The beads can be incubated directly with patient sperm to detect surface-bound antibody or can be incubated with normal donor sperm preincubated with serum cervical mucus or seminal plasma to detect anti-sperm antibodies in serum or genital-tract secretions. Both direct and indirect methods have a high degree of specificity and sensitivity and can distinguish the location of the antibody on the sperm head or tail. By using immunobeads coated with antibodies directed against different classes of immunoglobulin, the type of immunoglobulin can be determined.

Although serum has been frequently used for antibody studies, investigators have recently realized the need to examine reproductive-tract fluids or sperm for the presence of antibodies, as the serologic false-positive and negative rate, combined, is approximately 35%.[41] Because the failure to detect serologic anti-sperm antibodies on sperm or in genital-tract secretions may not be significant, a diagnosis of immunologic infertility should never be made on the basis of serology alone. For men, seminal plasma and, if possible, fresh sperm should be studied concurrently. For women, cervical mucus or follicular fluid should be evaluated. When antibodies are detected in a woman's genital-tract secretions, the specificity against her husband's sperm and donor sperm should be tested, as specific interactions occur in about 10%–15% of couples (Bronson RA. Personal communication).

ZONA-FREE HAMSTER-EGG PENETRATION ASSAY (ZFHPA)

The observation by Yanagimachi et al[42] that capacitated sperm from many species, including human beings, could fertilize hamster oocytes stripped of the zona pellucida represented a significant advance in evaluating sperm function. The ZFHPA measures the ability of the sperm to undergo the acrosome reaction, fuse with the egg vitelline membrane, undergo sperm-head decondensation, and form male pronuclei. Determination of nor-

mal penetration has been controversial, but in general, penetration rates <10%–15% indicate reduced fertility.[42–44] A positive result should be interpreted with caution as many more processes are involved in human egg fertilization, particularly in vivo, than are represented by the ZFHPA. The test is especially useful in providing an explanation for infertility in men with otherwise normal routine semen parameters.[44]

CONCLUSION

The development of better techniques for assessing sperm function has increased dramatically during the last few years, and as a consequence, spermatozoa physiology and pathology are becoming better understood. As techniques for diagnosing male infertility become more precise, our ability to devise treatments for patients with impaired fertility should be enhanced.

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