

Chapter 5

**Technologies for Assessing
Human Reproductive Function**

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Technologies for Assessing Human Reproductive Function

INTRODUCTION

One of the clearest indicators of reproductive health in a population is the incidence of healthy offspring. Birth statistics can be misleading, however, in their failure to indicate the number of couples who are not engaging in procreation or are unable to reproduce. Although individuals who wish to have children often take for granted their physical ability to do so, exposure to certain chemical, physical, or biological agents can compromise reproductive health and sexual functioning (chapter 4 describes the effects of individual agents).

Because of the structural and functional differences between the sexes, exposure to one of these agents may impair the reproductive capacity of one sex and not the other. Individual characteristics and lifestyle differences (e.g., smoking, age, nutrition) also alter sensitivity to some agents. Monitoring the reproductive health of individuals exposed to known or suspected reproductive hazards is thus an important step.

The following section describes the diagnostic procedures available to patients experiencing reproductive health problems. Although the emphasis is on infertility, it must be stressed that a thorough assessment of reproductive health includes factors not directly related to conception and fetal development (e.g., pubertal development, libido). Reproductive health refers to the entire composite of human reproductive and sexual functions and their integration with other organ systems (see chapter 3).

It is critical to note that an individual's reproductive competence cannot be verified in isolation. Fertility is the product of the specific interaction of a couple. Physical examination and laboratory analyses may determine that a man or woman is *potentially* fertile (i.e., sound reproductive organs, normal hormone levels, presence of reproductive cells), but fertility is verified only

after the couple has given birth to a healthy infant. Evaluation and treatment of infertility must therefore consider the couple as a unit. Ideal management is best achieved when the couple is seen together by a team of physicians (e.g., the man by a urologist, the woman by a gynecologist) (61).

Three features form the basis of a fertility evaluation in both men and women:

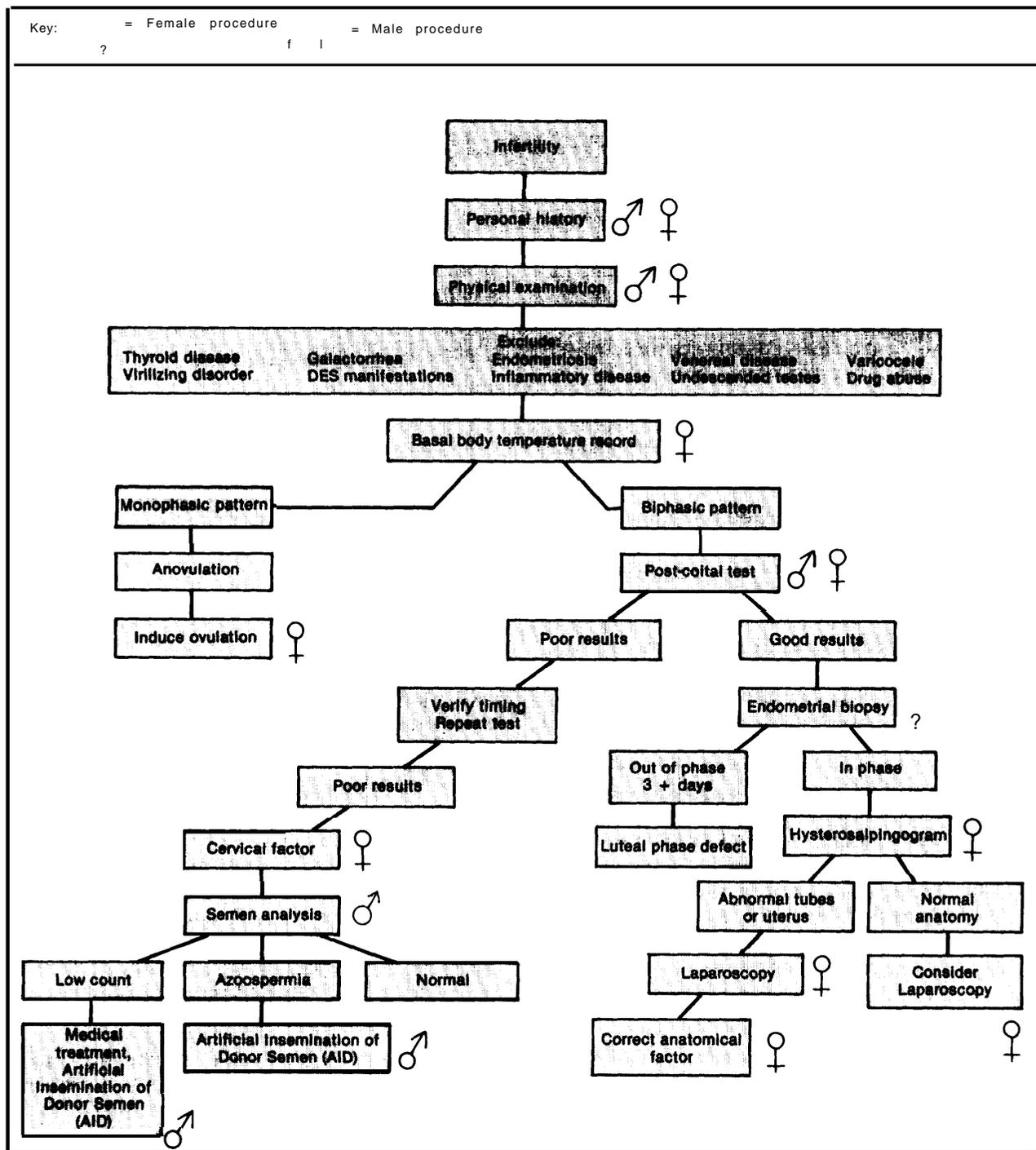
1. personal history (including medical, familial, occupational, and reproductive background);
2. physical examination; and
3. laboratory analyses (e.g., hormone studies, semen analysis, cervical mucus assays) (see figure 5-1).

Biological and practical considerations, however, demand that the parameters measured and the methods used be quite different for the two sexes. Whereas the male reproductive organs and germ cells (sperm) are readily accessible, the female correlates are not.

Physical examination of the male is simplified by the fact that his reproductive organs are external. Moreover, laboratory analysis of semen is a routine component of the male fertility evaluation. A man's ability to produce a semen sample and the analysis of various physical and functional properties of the sample provide an important indication of his reproductive health. These assays are safe, rapid, and easily performed with equipment standard to most hospitals and fertility clinics.

Unlike the male, the female reproductive organs are internal and her germ cells (eggs) do not leave her body. Direct observation of a woman's reproductive organs and germ cells, therefore, requires an invasive procedure or the use of special imaging equipment. The assessment of female reproductive competence thus relies heavily on indirect indicators. [An indirect indicator is defined here

Figure 5=1.—Chronology of Fertility Evaluation



SOURCE: Adapted from M. M. Seibel, "Infertility," in *Gynecologic Decision Making*, Emanuel A. Friedman (ed.) (Philadelphia: B. C. Decker Inc., 1983), pp. 68-69.

as one in which the reproductive endpoint under study is not observed, but its function is assumed based on the occurrence of related events. For example, while ovulation—the release of an egg cell from the female ovary—is not readily observable, there are associated changes in hormone levels and in body temperature that indicate when ovulation has occurred.] (A discussion of male and female reproductive function appears in chapter 3.)

Fertilization is but one of several events that are critical to successful reproduction. Others include transport of the fertilized egg to the uterus, implantation in the uterine wall, growth and development of the embryo/fetus, and delivery. Because each of the events subsequent to fertilization occurs within the female, the ability to accommodate and maintain a pregnancy is a component of female reproductive function.

As with other aspects of fertility, there is no absolute verification that can be made of a woman's ability to conceive and sustain a pregnancy, short of her actually doing so. However, several

clinical techniques enable the physician to monitor these events as they occur. These may prove useful in isolating the effects of various agents on the reproductive health of exposed individuals or on their offspring. In-utero monitoring of embryo/fetal development, for example, may detect the effects of agents that do not impede conception, but that elicit structural or functional abnormalities in the offspring of those exposed.

The following discussion examines methods for assessing human reproductive health, including events preceding, following, and independent of fertilization. These are the diagnostic techniques used with patients experiencing reproductive health problems. While diagnosis of the physiological basis of a reproductive disorder does not necessarily identify its source (e.g., workplace exposure, lifestyle characteristic), tracing patterns in the incidence of reproductive problems (e.g., infertility, deformed offspring) may make these correlations possible. The use of epidemiology and animal toxicology studies to identify reproductive hazards is discussed in chapter 6.

TESTS OF MALE REPRODUCTIVE HEALTH

The Fertility Evaluation

Personal History

Obtaining a thorough personal history is the first and one of the most important steps in a fertility evaluation. Information about the individual's personal and familial health background and the couple's sexual interaction can provide important insights into the cause of infertility. Certain drugs, medical procedures, and diseases, for example, can compromise reproductive function. Coital method (e.g., use of certain vaginal lubricants, timing, position) can also contribute to fertility problems, as can certain personal practices (e.g., frequent exposure to excessive heat as from saunas and hot baths). It is also important for the physician to ascertain whether the patient has experienced any form of sexual dysfunction (e.g., impotence or decreased libido), whether the couple engages in intercourse during the woman's ovulatory period, at which time she is most likely

to conceive, and whether the male has successfully fathered healthy children with his present or any previous mate. Table 5-1 outlines the components of a thorough personal history questionnaire. In addition, a sample personal history questionnaire is shown in appendix A.

Physical Examination

A careful physical examination is critical to the fertility evaluation. This includes examination of the secondary sex characteristics (e.g., hair distribution, breast development), and of cardiovascular and neurologic function (e.g., strength of pulse in lower extremities, reflexes, pelvic sensation), as well as of the genitals. The presence and structural adequacy of the various components of the genital tract (e.g., vas deferens, prostate, epididymides) must be verified. Particular structural abnormalities associated with impaired fertility are sought (e.g., hernia, varicocele—

Table 5-1.—Patient History

Sexual history

Duration of sexual relations with and without birth control.
Methods of birth control.

Sexual technique: penetration, ejaculation, use of lubricants (some are spermicidal).

Frequency and timing of coitus. Does it coincide with ovulation?

Past marital history of both partners, including pregnancies and miscarriages.

Past history: male

Developmental: **age** of testicular descent, age of puberty, history of prepubertal obesity, gynecomastia (excessive breast development), congenital abnormalities of urinary tract or central nervous system.

Surgical: orchiopexy (surgical placement in the scrotum of an undescended testis), pelvic or retroperitoneal (behind the abdomen) surgery, herniorrhaphy (surgical repair of a hernia), sympathectomy (interruption of sympathetic nervous system pathways), vasectomy, injury to genitals, spinal cord injury.

Medical: urinary infections, venereal disease (including non-specific urethritis), mumps, renal disease, diabetes, radiotherapy, recent allergic febrile (fever-inducing) or viral illness (may affect semen quality), epididymis, tuberculosis, smallpox (causes obstructive azoospermia) or other chronic diseases, anosmia (absence of sense of smell), midline defects.

Drugs: complete list of all past and present medications. Many drugs may interfere with spermatogenesis, erection, ejaculation.

Occupation and habits: exposure to chemicals and heat, hot baths, steam baths, radiation, biological agents, physical exertion, cigarettes, alcohol, diet, other habits.

Sexual: libido, erectile capacity, ejaculatory capacity, position during coitus.

Past marital history of both partners: any offspring with other partners.

Previous infertility evaluations and treatments.

Past history: female

Developmental: age at onset of menstruation, age at development of secondary sex characteristics (e.g., breast development), congenital abnormalities of central nervous system.

Surgical: pelvic operations, appendectomy,

Medical: tuberculosis, venereal disease, endometriosis (aberrant appearance of uterine-like tissue in various locations in the pelvic region), tumors, menstrual irregularities, diabetes, other chronic diseases.

Menstrual: regularity of menstruation, length of menstrual cycle, number of days of menstrual bleed per cycle, premenstrual symptoms (e.g., pain, water retention).

Contraception: present and past methods.

Obstetrics: full-term deliveries, pregnancy complications, abortions, premature deliveries, previous infertility.

Drugs: complete list of all past and present medications.

Occupation and habits: exposure to chemicals, radiation, biological agents, physical exertion, cigarettes, alcohol, diet, other habits.

Sexual: libido, orgasm capacity, position during and after coitus.

Family history

Blood disease

Cancer

Congenital defects

Endocrine disorder

Genetic disease

Heart condition

Impaired offspring

Kidney disease

Necrologic disorder

Reproductive disorder

SOURCE: Adapted from: R. J. Sherins and S. S. Howards, "Male Infertility," *Campbell's Urology*, J. Hartwell Harrison, Reuben F. Gittes, Alan D. Perlmutter, Thomas A. Stanley, and Patrick C Walsh (eds.) (Philadelphia: W. B. Saunders Co., 1978), pp. 715-776; and J R Wilson, "infertility," *Obstetrics and Gynecology* William J. Ledger (ed.) (St. Louis, MO: C. V. Mosby Co., 1983), pp. 160.174.

varicose veins in the testes, hypospadias-opening of the penis on the underside.) In addition, the size and volume of the testes are measured, as testicular atrophy is an indication of reduced sperm supply (3,22,59). (See figure 5-2.)

Physical examination of a patient who describes problems with impotence may include an assessment of erectile capacity. Determining the occurrence of erections during sleep (nocturnal penile tumescence—NPT) is considered one of the best means for distinguishing between physiologic and psychogenic causes of sexual dysfunction (64). NPT monitoring may be done in a laboratory or at home. The principle of the monitoring device is the same in either case: a strain gauge worn around the penis indicates changes in penile cir-

cumference during sleep. * (See figure 5-3.) While monitoring devices used at home are less precise and cannot measure certain other relevant factors (e.g., duration of erection, correlation with REM sleep cycles), some physicians find that they provide a sufficient indication of nocturnal erectile function for most patients. The cost of the home monitoring device is significantly lower than that of laboratory monitoring (i.e., \$15 as opposed to \$1,500) (64).

● One simple home monitoring method uses postage stamps to measure NPT. Torn perforations in a ring of stamps worn during sleep indicate nocturnal erection.

Figure 502.- Diagnostic Techniques in Fertility Assessment

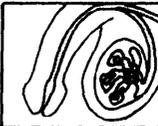
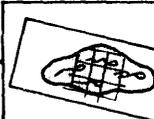
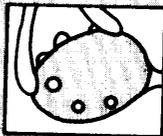
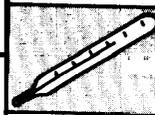
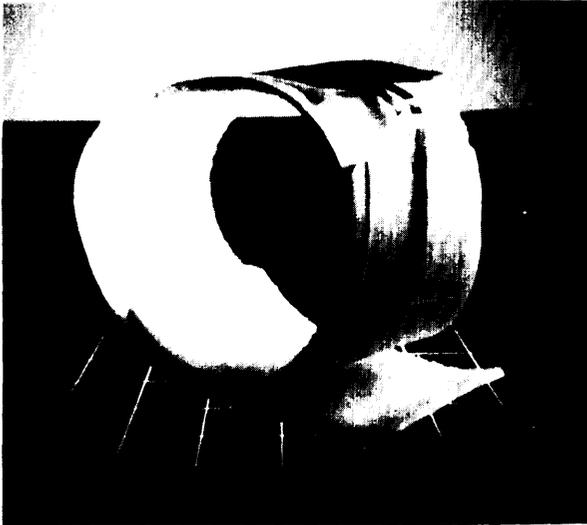
Parameter		Test	
Male			
 <p>Genitals: Physical abnormalities can impair spermatogenesis and/or ejaculation.</p>		<p>Scrotum: Palpation may detect structural abnormalities of the testes, vas deferens, or epididymides. Testicular size and volume are also measured.</p>	
			<p>Prostate: Tenderness at palpation indicates infection.</p>
			<p>Varicose veins: Enlarged veins in scrotum may increase temperature above favorable sperm production conditions.</p>
			<p>Hypospadias: Opening on the underside of the penis impedes deposition of sperm in the vagina.</p>
			<p>Vasography: Instillation of dye followed by X-ray imaging of the ejaculatory tract discloses any obstruction.</p>
 <p>Sperm: Abnormal sperm production, structure, or activity can impede union with egg.</p>		<p>Semen analysis: Assesses appearance and pH of seminal fluid, and sperm shape, concentration, and motility.</p>	
			<p>Penetration assays: Tests to evaluate sperm's ability to travel through cervical mucus and penetrate an egg.</p>
			<p>Hormone assays: Verify circulation of hormones necessary for the entire range of reproductive functions.</p>
			<p>Immunologic testing: Diagnosis of male blood or seminal fluid for antibodies that can incapacitate sperm.</p>
			<p>Testicular biopsy: Tissue cut from inside testes indicates if sperm are being produced.</p>

Figure 5-2.—Diagnostic Techniques in Fertility Assessment—Continued

Parameter	Test
<p style="text-align: center;">Female</p>  <p>Ovaries: Ovary fails to release egg or releases it in an irregular cycle.</p>	 <p>Body temperature: Woman's body temperature taken on waking every day. A rise in temperature may indicate ovulation.</p>  <p>Hormone assays: Normal ovarian activity is reflected by timed shifts in blood/urine hormone concentrations throughout the menstrual cycle.</p>  <p>Endometrial biopsy: Tissue scraped from lining of uterus can reveal the influence of ovarian hormones, verifying ovulation.</p>
 <p>Fallopian tubes: Can be blocked or scarred by infection or endometriosis, or abnormal growth of uterine tissue outside the uterus.</p>	 <p>Hysterosalpingogram: X-ray traces iodine dye through cervix and fallopian tubes to uterus to detect tubal obstruction or uterine irregularity.</p>  <p>Laparoscopy: Fiberoptic scope, inserted into abdomen beneath the navel, may reveal scar tissue, cysts, or endometriosis.</p>
 <p>Uterus: Abnormally shaped uterus, scarring, or abnormal growths can prevent sperm from reaching the egg or fertilized egg from implanting.</p>	 <p>Ultrasonography: Noninvasive imaging technique allows visualization of female reproductive organs on video screen.</p>  <p>Hysteroscopy: Fiberoptic scope inserted through the cervix allows the uterus to be viewed.</p>
 <p>Cervix: Physical abnormalities or immunity make the cervix unreceptive to sperm.</p>	 <p>Cervical mucus analysis: Thick, impenetrable, or acidic mucus may impair sperm motility or viability.</p>  <p>Post-coital test: Examination of cervical mucus several hours after intercourse checks sperm survival and motility in the cervix.</p>  <p>Immunological testing: Diagnosis of female blood or cervical mucus for antibodies against sperm that can impede motility.</p>

SOURCE: Adapted by permission of M. E. Challinor and Susan Arritt for *Science 85 Magazine*. Copyright 1985 by the American Association for the Advancement of Science.

Figure 5-3.—A Gauge Used to Measure the Occurrence of Erection During Sleep



Diameter of gauge is approximately 1.5 inches

SOURCE *Medical World News* 25:47-52, 198

Laboratory Evaluation

Examination of the male experiencing reproductive difficulties may include one or more of the following laboratory procedures:

- Semen analysis Evaluation of semen is one of the cornerstones of the infertility examination. A variety of procedures can be used to assess the structural and functional characteristics of the patient's sperm and seminal fluid.
- Hormone assay. Where semen analysis repeatedly shows abnormalities, hormone assays may inform the physician about the source of the difficulties. (See Tech. Note 1.) The proper balance of hormones in the blood is critical to the entire range of reproductive functions.
- Urinalysis and urine culture. These screen for urinary tract infections or disorders that might hamper reproductive function.

Because of the prominence of semen analysis in examination of the male fertility patient, the following discussion examines the components of a standard semen analysis and their relevance to reproductive and sexual function.

Semen Quality

Several physical characteristics of semen have been associated with male reproductive competence. These include:

- ejaculate appearance,
- ejaculate pH,
- ejaculate volume,
- sperm density,
- sperm motility,
- sperm vitality, and
- sperm morphology.

Researchers in the field disagree as to which of these endpoints most significantly effects male fertility. There is presently no definitive indication that any single factor is the most important. Rather, it appears that they operate together in determining the reproductive competence of each individual (15,18,19).

In addition, there is no broadly accepted definition of what constitutes "normal semen." Laboratories differ in what they designate as the critical level for each semen characteristic (e.g., the number of motile or morphologically normal sperm, the rate of forward progression). Several factors contribute to these disparities:

- The quality and quantity of semen vary significantly among all men, even among fertile men.
- Each individual is subject to normal fluctuations in semen quality and quantity. Age, seasonal change, illness, and ejaculation frequency are among the factors known to induce these shifts. *
- Many of the measurements included in a semen analysis are subjective, qualitative judgments. This makes comparison of data from different laboratories and/or different clinicians difficult.
- Proper collection and diagnostic techniques are critical. Accuracy of findings may be compromised if the specimen is collected incorrectly, not analyzed promptly, or mishandled in any way.

*Because of the fluctuations in semen quality and the potential for laboratory error, a minimum of three semen samples is usually recommended. An interval of at least 10 days between samples, with sexual abstinence for 2 to 4 days preceding sample collection, is optimal (61).

- The methods and standards for statistical analysis of semen quality data vary, making comparison of results from different studies difficult.

Although efforts are being made to develop methods that will standardize and objectively measure these parameters, the time, expense, and amount of equipment they require are as yet beyond the means of many laboratories and clinics. Thus, while semen analysis remains an important aspect of a fertility examination, there are no absolute values associated with any of the physical characteristics that are assessed.

Ejaculate Appearance

Several physical properties of healthy semen make evaluation of ejaculate appearance an important step in the assessment of semen quality:

- Freshly ejaculated semen is a white, yellow, or gray fluid that coagulates at the time of ejaculation.
- Enzymes produced by the prostate gland cause the semen to liquify 3 to 25 minutes later. Semen viscosity is, therefore, a measure of secretory activity and enzymatic function of the prostate and seminal vesicles (61).
- A high incidence of agglutination (head-to-head, head-to-tail, or tail-to-tail clumping) among the spermatozoa in a sample may indicate the presence of infection or of anti-sperm antibodies in the seminal fluid. (See Tech. Note 2.) An observation of greater than 10 percent agglutination in a sample is considered abnormal (19,23). (See figure 5-4.)

Ejaculate pH

Normal semen pH is 7 to 8. A low pH may be the result of a contaminated sample or may indicate obstruction of the ejaculatory ducts (61).

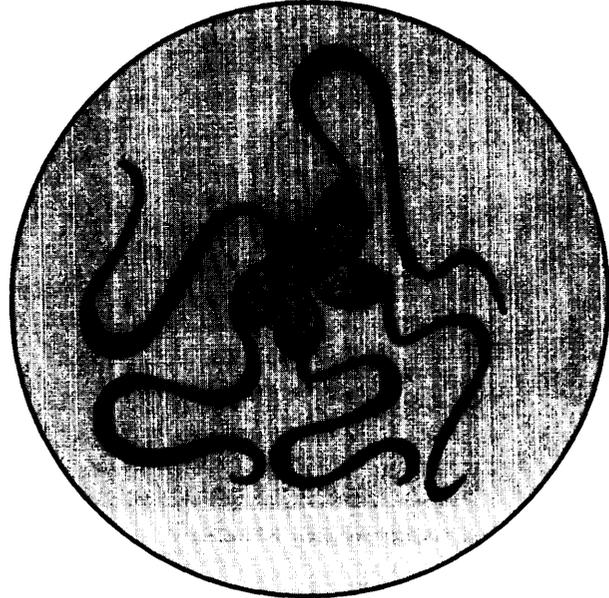
Ejaculate Volume

The amount of semen in an ejaculate normally ranges from 2.5 to 5 milliliters (19).

- Smaller volumes may indicate functional deficiencies of the prostate and/or seminal vesicles, or incomplete collection (19,23).

Figure 5-4.—Sperm Agglutination

Sperm clumping head to head (agglutination)



SOURCE: Reprinted from *You Can Have a Baby*. Illustrations by Delia Malone. Copyright © 1985 by Joseph H. Bellina, M.D., and Josleen Wilson. Used by permission of Crown Publishers, Inc.

- Excessive ejaculate volumes may be the result of a long period of abstinence prior to the test procedure.

Where abnormal ejaculate volumes are obtained, the test should be repeated to differentiate faulty collection technique from physiological impairment.

Sperm Density

Sperm density refers to the number of sperm per milliliter of semen. Microscopic observation enables these counts to be made. Automated techniques are also available (7 D).

Despite the relative ease and objectivity with which sperm density can be measured, there remains no uniformly accepted specification of the number of sperm per milliliter of semen necessary to establish fertility (23,71). (See Tech. Note 3.) Two factors contribute to this uncertainty:

1. Total semen volume and number of sperm per ejaculate differ among all men, even among fertile men. There is no sperm con-

centration threshold—except zero—below which a man is absolutely infertile.

- Each male is subject to natural fluctuations in sperm concentration (71). Age, seasonal change, illness, and ejaculation frequency are among the factors known to induce shifts in this parameter (23).

Because of sperm density variability, at least three semen samples must be analyzed before concluding that a man is azoospermic, * oligospermic, ** or normal (61). Where few or no sperm are ejaculated and normal hormone levels have been confirmed, the physician must determine whether the absence of sperm is due to impaired sperm production or to obstruction of the ejaculatory ducts. Testicular biopsy (extraction and microscopic observation of testicular tissue) and lasography (X-ray of the seminal transport system) are the two diagnostic procedures used for these purposes (59). (See Tech. Note 4.)

Sperm Motility

The importance of sperm motility in establishing male fertility is well documented (15)18,23, 33,50,70). There is a strong correlation between motile sperm and successful fertilization (7,46). No precise data on the levels of motility necessary to establish fertility are available, however, because this parameter is difficult to measure accurately and objectively (23,71).

Several factors contribute to the difficulties in defining specific levels of sperm motility necessary to establish fertility:

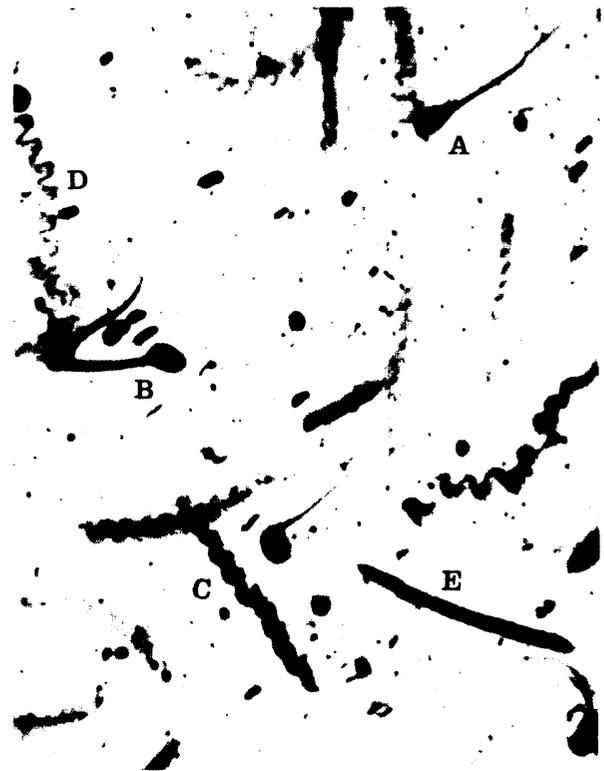
- The sensitivity of sperm motility to temperature and to time between collection and measurement limits the comparability of data from different laboratories, where collection procedures may vary (71),

The extreme subjectivity of the visual rating system commonly used in motility assessment makes comparison of motility data from different laboratories problematic. (See Tech. Note 5.)

Despite these difficulties, recognition of the significance of sperm motility in relation to fertility has inspired efforts to develop precise, objective measures of this parameter (23). These encompass a range of photographic and automated techniques through which overall sample motility and individual sperm velocities may be determined. (See Tech. Notes 6-8 and figure s-5.)

While each of these techniques offers increased objectivity and accuracy in the measurement of sperm motility, the equipment, time, and expense they require limit their clinical applicability (50), and standard clinical tests of sperm motility remain of limited predictive value with regard to fertility (71).

Figure 5.5.—Sperm Movement Patterns



A) Immotile spermatozoon; B) stationary spermatozoon with active flagellum; C) roiling spermatozoon; D) yawing spermatozoon; E) straight-swimming spermatozoon exhibiting neither rolling nor yawing.

SOURCE: J. W. Overstreet, D.F. Katz, F. W. Hanson, et al., "A Simple Inexpensive Method for Objective Assessment of Human Sperm Movement Characteristics," *Fertility & Sterility* 31:162-172, 1979. Reproduced with permission of the publisher, **The American Fertility Society**.

• Azoospermia is the complete absence of sperm.

* Oligospermia refers to extremely low levels of sperm production

Sperm Vitality

A dye that selectively stains dead cells permits the ratio of live to dead spermatozoa in a sample to be determined. This technique is particularly useful in semen samples showing low levels of motility because it enables differentiation between immotile and dead sperm (19,23).

Sperm Morphology

The natural diversity of sperm shape and size among both fertile and infertile men makes it difficult to define "normal sperm morphology." while the prototypical human sperm is characterized as having an oval head, estimates of its dimensions and of the percentage of sperm that must be of this ideal morphology in order to achieve fertility are disputed by fertility experts.

The subtlety of the structural variations among sperm further complicates efforts to categorize the cells. Judgments are qualitative and subjective, limiting comparisons of morphology data from different laboratories, and making it difficult to determine the precise relationship of sperm morphology to fertility (15,18,23).

Recent efforts to standardize these measurements include the use of:

- reference slides (70);
- morphology overlays (36); and
- direct morphometric measurement (i.e., length, width, area, circumference) (35,58).
(See Tech. Note 9 and figures 5-6 and 5-7.)

However, no morphology assessment technique completely eliminates the role of human decision and human error in evaluating this parameter. It remains difficult to define specific criteria for the shape, size, and percentage of "normal" sperm necessary to establish male fertility.

Despite these difficulties, there is substantial evidence for the importance of sperm morphology in establishing male reproductive capacity (36, 70), * Although subjective, evaluation of sperm morphology remains an important component of semen analysis.

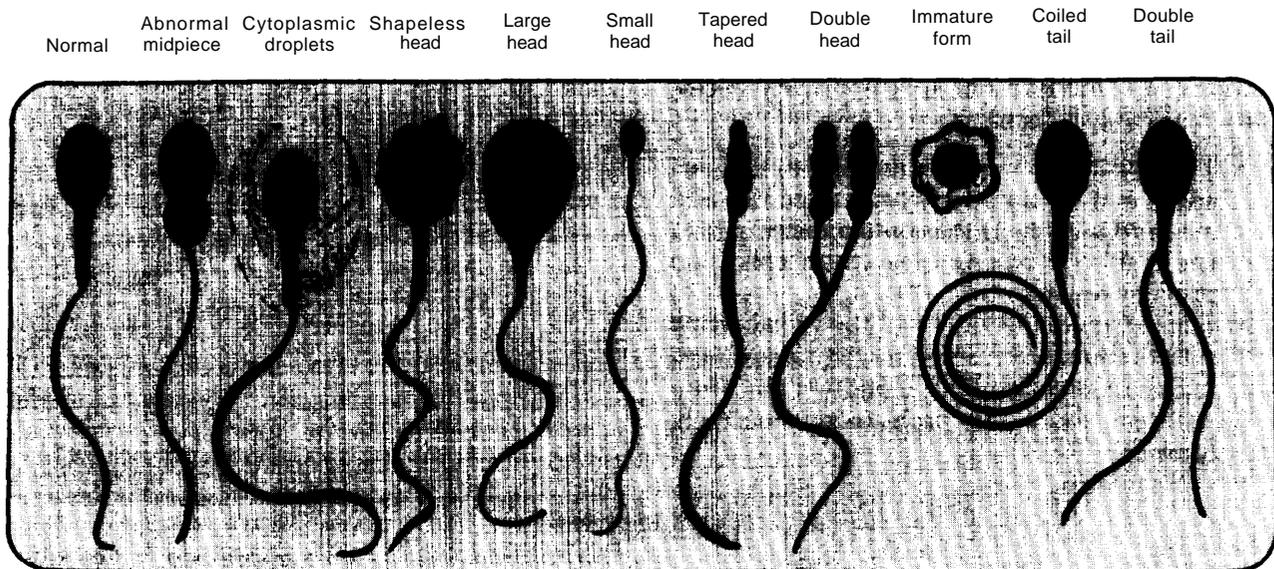
Sperm Function

Sperm Function

Because tests of semen quality have failed to provide specific, reliable criteria by which to assess male reproductive capacity, researchers are

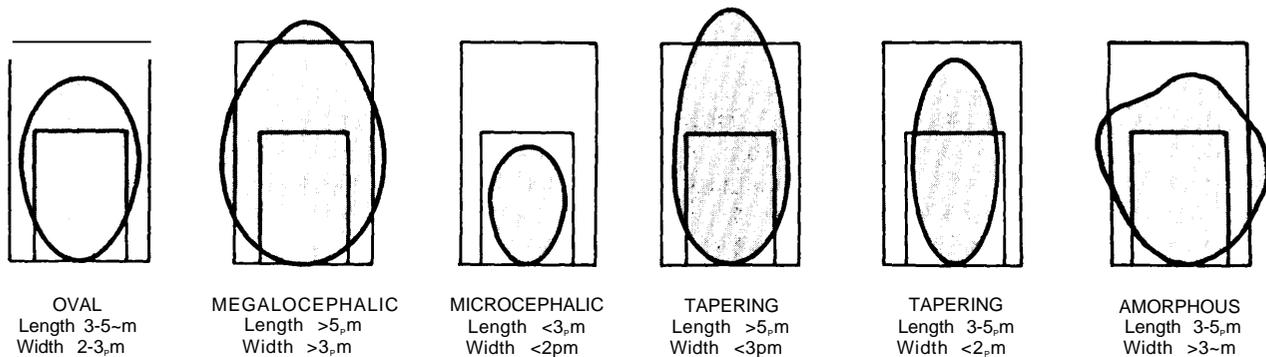
- Studies show that morphologically abnormal sperm are poorly or nonmotile, making these misshapen cells less viable (17,36,43,52).

Figure 5-6.-Sperm Morphology: Some Categories



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Figure 5.7.—The Morphology Overlay



Base of sperm is aligned with the bottom of the overlay. If the length and width lie between the two boxes, the classification is oval or "normal" morphology.

SOURCE: D. G. Katz, L. Diel, and J. W. Overstreet, "Differences in the Movement of Morphologically Normal and Abnormal Human Seminal Spermatozoa," *Biology of Reproduction* 26:566-570, 1982.

seeking alternative methods. These emphasize the functional ability rather than the physical characteristics of the sperm cells (1,23). While the ultimate evidence of normal sperm function is conception, the following section describes two tests that may be of predictive value.

Cervical Mucus Penetration

In order to reach and fertilize an egg cell, a sperm must migrate from the vagina through the female endocervical canal (the pathway from the **vagina to the** uterus). Its ability to penetrate the cervical mucus that fills this area is an important determinant in its successfully accessing the egg.

There are several laboratory techniques for the evaluation of sperm-cervical mucus interaction. (See Tech. Note 10.) Each examines the ability of the sperm to penetrate the mucus and the vitality of the sperm after penetration (i.e., some sperm may penetrate but thereafter become immobilized) (12,38,39,65),

A significant caveat of the test is the variability of cervical mucus:

- Normal changes in mucus quality occur throughout the menstrual cycle (23). As a result, a woman's mucus may resist her husband's sperm in one test, and be easily penetrated in a subsequent study (5).
- The mucus of different women, even women at the same stage of their cycle, varies in its

receptivity to sperm, making it difficult to establish specific, broadly applicable criteria for mucus penetration in relation to fertility (19)23).

In order to account for these differences, it is useful to do a cross-study of both the semen and the cervical mucus with control samples. (See Tech. Note 11.) This enables the physician to determine whether the couple's fertility problem is attributable to one of the two partners or is the result of a compatibility problem.

Where a compatibility problem is suspected, it may be useful to check for the presence of anti-sperm antibodies. Antibodies can occur in the male—autoimmunity—or in the female—sperm allergy. They may be present in the blood serum and/or the reproductive tract of either individual and can cause varying degrees of reproductive impairment, from reduced fertility to infertility. Various techniques enable the detection of antisperm antibodies in the blood, semen, and cervical mucus. The latter presents the most difficulty, making confirmation of antisperm antibodies in the female reproductive tract problematic (9). (See Tech. Note 12.)

Sperm-Oocyte Interaction

For fertilization to occur, a single sperm cell must succeed in penetrating a female egg cell. Ethical considerations bar laboratory experimen-

tation with human sperm penetration of live human eggs (1). Two alternative techniques have been developed:

1. The more common of the two techniques—the zona-free hamster egg penetration test—monitors the interaction of human sperm with hamster eggs (72). Its reliability as a definitive measure of male fertility, however, remains uncertain. (See Tech. Note 13.)
2. Recognizing the weaknesses of the hamster egg test, an alternative approach observes the interaction of human sperm with non-

living human egg~ This technique is not yet widely available because of logistical difficulties in obtaining a supply of human eggs.*

Difficulty in interpreting sperm-egg interaction tests persists because the percentage of sperm that successfully penetrate a test egg varies significantly, even among fertile men. There is no universally accepted definition of what constitutes “normal sperm penetration.” (See Tech. Note 13.)

*The eggs are obtained from the ovaries of women undergoing elective surgery (8,20,49).

TESTS OF FEMALE REPRODUCTIVE HEALTH

Because of the relative inaccessibility of the female reproductive organs and their contents, assessment of female reproductive function relies heavily on inferential and indirect observations. An indirect indicator is defined here as one in which the reproductive endpoint under study is not observed, but its function is implied by the occurrence of related events. Menstrual regularity is an example. It signifies the presence of oocytes (egg cells) and the ability of the hypothalamic-pituitary-gonadal axis (the hormonal feedback system) to coordinate ovulation (the release of an egg cell from the ovary to the uterus), while none of these events is actually observed. (A discussion of female reproductive function appears in chapter 3.)

This section describes direct and indirect measures of the parameters that are assessed in a female fertility evaluation. These include:

- Personal history Obtaining comprehensive information about a patient’s medical, familial, occupational, and reproductive history is the first step in a fertility evaluation.
- Secondary sex characteristics: The attainment of pubertal milestones and normal development of secondary sex characteristics (e.g., breast development, hair distribution) are an indication of hormone secretion and response.
- Ovarian function: The female ovary becomes functional at the time of reproductive

maturity. Events that are associated with the monthly menstrual cycle denote ovarian activity and are important indications of female reproductive health.

- Cervical mucus: Secretion of cervical mucus is fundamental to the female reproductive cycle. The receptivity of a woman’s mucus to sperm is an important determinant of her ability to become pregnant.
- Endometrial cells: Accommodation of pregnancy necessitates thickening of the uterine wall. The appropriate growth response of the cells lining the uterus (endometrial cells) to monthly hormonal secretions is an important determinant of fertility.
- Tubal patency/uterine structure The structural health of the fallopian tubes and uterus is necessary for the establishment of pregnancy as a fertilized egg must travel through the tubes before implanting in the uterine wall. While no methods currently enable monitoring of gamete transport, fertilization, zygote transport, or implantation, verifying the structural health of the fallopian tubes and uterus indicates the potential for these events to occur.

Assessment of these factors provides an indication of a woman’s capacity to conceive. However, because the events that succeed fertilization in the reproductive process occur within the female, there are additional aspects of female re-

productive competence that must be considered. These include:

- **Implantation/establishment of pregnancy.** Pregnancy is established when a fertilized egg implants in the uterine wall. While neither fertilization nor implantation are observable events, hormonal secretions provide an indication of their occurrence.
- **Embryonic differentiation and fetal development.** The ability of the female reproductive system to sustain a pregnancy and the normal development of the fetus in utero can be assessed through a variety of techniques.
- **Delivery and lactation.** These are significant aspects of female reproductive function that may be assessed to determine effects of toxic exposure.

Personal History

Obtaining information about a woman's medical, occupational, familial, and reproductive history is the critical first step in a fertility evaluation. Information obtained in this initial stage of the examination can provide important insights into the source of fertility problems. A history of menstrual irregularity, pelvic inflammatory disease, or surgery, for example, indicates a possible physiological or anatomical basis for infertility, while questions about coital frequency and technique may indicate that these are the source of the couple's inability to reproduce.

Table 5-1 lists aspects of medical, occupational, personal, and familial history that are important to a fertility assessment. The table reflects the importance of considering the infertile couple as a unit. A more detailed description of pertinent information to be obtained from the infertile couple is provided by the sample history questionnaire in appendix A.

Physical Examination

Physical examination of the fertility patient seeks evidence of physiological and/or anatomical bases for infertility. Standard health parameters (e.g., height, weight, blood pressure) and neurologic function (e.g., reflexes, pelvic sensation) are measured, and particular attention is paid to any anatomical abnormalities.

While the gonads are not external in the female as they are in the male, secondary sex characteristics (i.e., breast development, hair and fat distribution) are observable and provide an important indication of hormonal secretion and response. Excessive facial and/or body hair, for instance, may be the result of androgenization (an excess of male hormones in a female).

A standard pelvic examination, including inspection and palpation of structures throughout the genital tract, may isolate infection, tumors, adhesions, or other abnormalities contributing to reproductive difficulties.

If this initial examination fails to isolate the source of infertility, the physician undertakes a more detailed evaluation of the patient's reproductive capacity. The following section describes the specific parameters measured and the methods used. (For a summary of the diagnostic techniques used, see figure 5-2.)

Ovarian Function

Although the female genital tract and the primordial germ cells (the cells that develop into egg cells) are developed prenatally, ovarian activity first becomes apparent with the onset of menstruation at puberty. Consequently, damage sustained as a result of prenatal toxic insult may go unnoticed for the first 12 to 16 years of a girl's life (44,45).

The specifics of oocyte (egg cell) development are described in chapter 3, but it is important to note that there are fundamental differences between the production of female germ cells (eggs) and the production of male germ cells (sperm). Because of these differences, exposure to agents that are toxic to reproductive cells has different consequences for women than for men.

In a female fetus, all primordial germ cells progress to the oocyte stage before birth. There is no further generation of oocytes. An agent that is toxic to oocytes thus depletes a finite supply. A male, by contrast, continues to generate spermatogonia—the analog of the female oocyte—after he reaches reproductive maturity. From puberty onward, sperm cells are continuously produced from spermatogonia in a process that takes between 64 and 74 days. Contami-

nated spermatogonia are thus effectively “washed” from the system and replaced by fresh cells. This “cleansing” is obviously ineffective if exposure to the toxin continues or if there is chromosomal or hormonal damage that prevents the generation of healthy sperm (44).

Proper function of the developed ovary requires coordination of the hypothalamic-pituitary-gonadal axis; i.e., the correct balance of hormones must be present and the reproductive organs must have the capacity to respond to hormonal activity. The system relies on continual feedback mechanisms that signal increases and decreases in the production of particular hormones. Fluctuations in the concentration of these substances, in turn, cue the events of the menstrual cycle. Successful coordination of the hormonal and growth activities results in ovulation, the maturation and release of one egg cell approximately every 28 days (4,44). (See Tech. Note 14.)

Indirect Indicators

Regular Menstruation.—A regular menstrual cycle is one of the best indicators that the hypothalamic-pituitary-gonadal axis is functioning properly. Correct shifts in hormone concentration, the formation of the follicle complex (containing the oocyte to be released), the growth of this complex, and the ultimate release of the mature egg cell for passage to the uterus are all implied by the occurrence of menstruation (45). (See Tech. Note 14.)

Hormone Levels.—Hormonal feedback mechanisms are critical to ovarian function. Hormones produced outside of the ovary serve to stimulate the organ’s production of additional hormonal substances. Each is necessary in order for the events in the monthly cycle (e.g., maturation and release of an egg, uterine tissue growth) to occur. Verifying the proper balance of and shifts in the levels of these substances, therefore, serves as a strong indicator of ovarian function. Blood serum and/or urine may be assayed for information on each of the relevant hormones. In addition, a new method permits levels of one key hormone, progesterone, to be monitored in saliva (74). (See Tech. Note 15 and figure 5-8.)

Basal Body Temperature.—Normal fluctuations occur in a woman’s resting body temperature throughout her 28-day cycle. Basal body temperature is thus a valuable indirect measure of ovarian activity. The temperature shifts can be measured and recorded by the woman herself with a standard oral or rectal thermometer. (See Tech. Note 16, figures 5-8 and 5-9.)

Cervical Mucus.—Cervical mucus fills the cervical canal, the pathway from the vagina to the uterus. Samples can be collected quickly and easily from the cervical opening. The quality and quantity of this mucus change over the course of the menstrual cycle in accordance with estrogen fluctuations. As a result, assessment of mucus quality and quantity indicates a woman’s menstrual phase. (See Tech. Note 17, figures 5-8 and 5-10.)

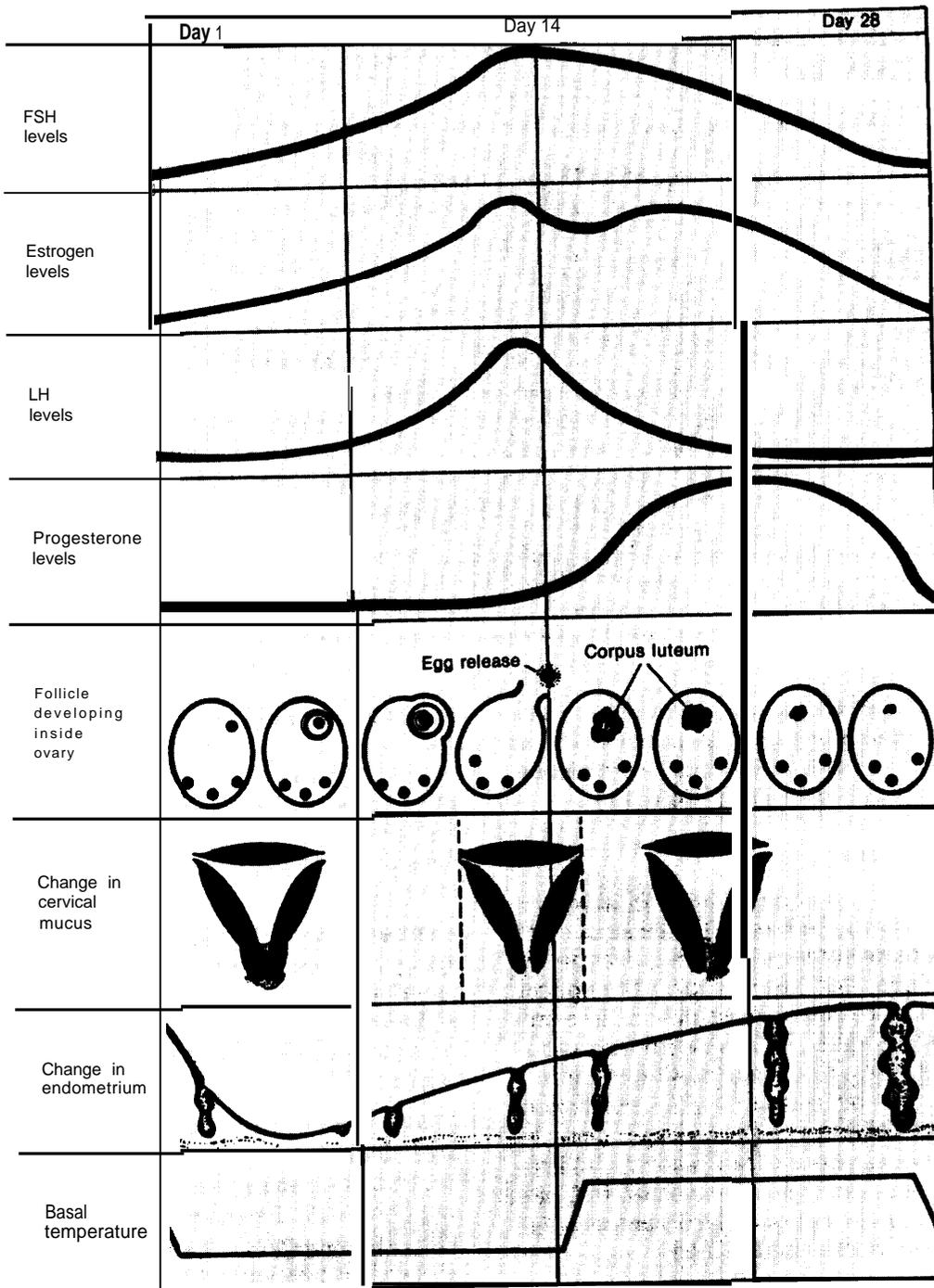
The changes that occur in conjunction with ovulation make it the only time of the menstrual cycle during which the mucus is penetrable by sperm. Observation of cervical mucus quality, particularly preovulatory mucus, is thus important in assessing female fertility (32,68).

Timed Endometrial Biopsy Adequacy of Luteal Phase.—Endometrial samples (tissue from the uterine wall) provide good evidence of ovarian activity and of the adequacy (length) of the luteal phase. The luteal phase, the portion of the menstrual cycle that occurs between ovulation and menses, is characterized by a thickening of the uterine wall tissue. A typical luteal phase is precisely 14 days long. Deviation indicates a luteal phase deficiency (32,56,67). (See Tech. Note 18, figures 5-8 and 5-9.)

Timed endometrial biopsy is the standard means of identifying a luteal phase deficiency. * The procedure examines tissue from the endometrium (uterine wall). Because thickening of the endometrium is a regular occurrence of the menstrual cycle, a woman’s menstrual stage may be determined based on the development of her endome-

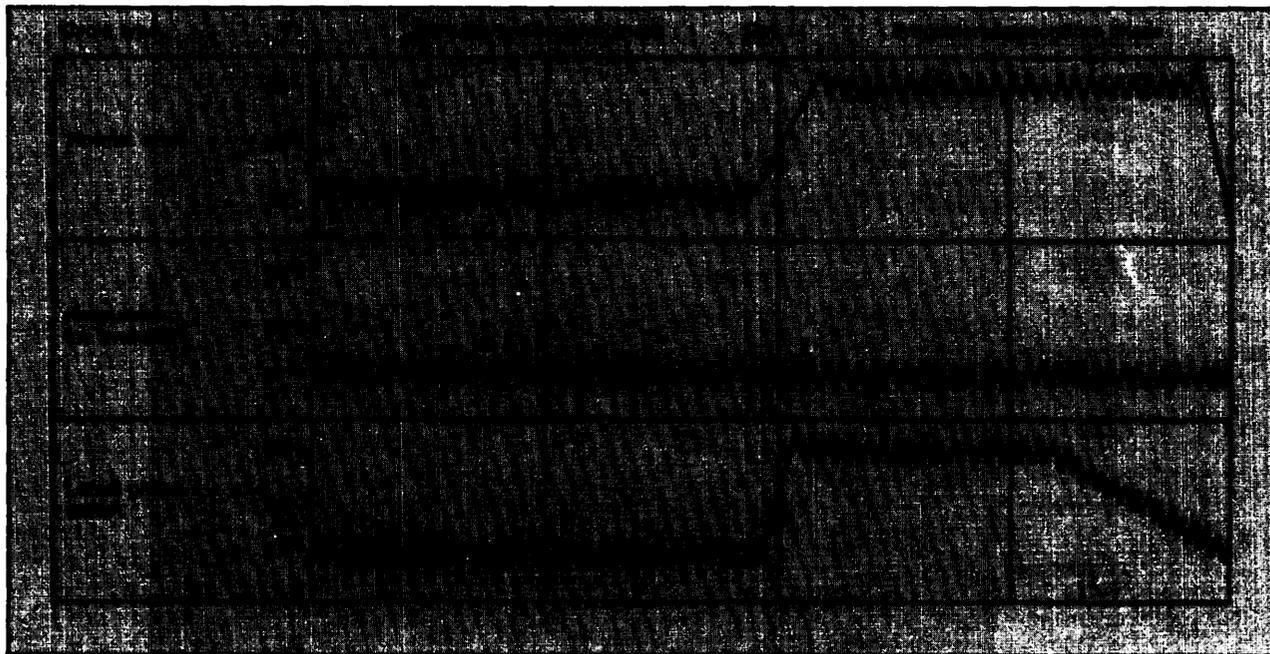
● Progesterone levels also indicate luteal adequacy. This hormone, secreted after ovulation, stimulates endometrial cell growth during the luteal phase. Progesterone concentrations may be monitored in blood serum and urine assays. An alternative method measures progesterone concentrations in saliva. (See Tech. Note 15a.)

Figure 5-8.—The Menstrual Cycle



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Figure 5-9.—Basal Body Temperature Patterns Throughout the Menstrual Cycle



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trial cells, and the date of her next menses may be predicted. If menses occurs sooner or later than the expected date, a luteal phase deficiency is identified (56,67). (See Tech. Note 19.)

Direct Indicators

Laparoscopy.—Direct observation of an ovary to determine whether it has sustained structural damage or is deficient in oocytes requires an invasive procedure. The laparoscope is the optical instrument used to detect gross defects in ovarian structure (e.g., cysts, lesions). With the instrument inserted through a small incision in the abdominal wall, the ovaries are visible. Because it is an invasive procedure, laparoscopy is usually undertaken as a measure of last resort, when reproductive organ damage is suspected but has been unidentifiable with standard clinical diagnostic techniques. (See figure 5-I.)

Laparoscopic Ovarian Biopsy.—Observation of egg cells within the ovaries requires removal and microscopic observation of ovarian tissue. Tissue samples are taken with the laparoscope in place. While laparoscopic ovarian biopsy is a

surgical procedure, it is the only means by which the contents of the ovaries can be directly observed. By viewing the tissue sample under a microscope, the presence of oocytes and of growing follicles as well as the health of the ovarian cells themselves can be verified (45). (See Tech. Note 20.)

Ultrasonography.—Ultrasonography is an imaging technique by which ovarian activity can be monitored. The projection of sonic waves into the abdominal region and the diagnosis of the wave reflections allows “visualization” of the underlying organs.

No adverse effects of ultrasonography have been demonstrated in humans. Moreover, most hospitals and many physicians have the equipment necessary for the procedure. Ultrasound imaging thus appears a safe and convenient means to monitor ovarian activity, including follicular growth and ovulation (4,12). While clinical use of ultrasound for ovarian imaging is limited, the technique is widely used as a means of fetal imaging during pregnancy. (See *Embryonic Differentiation and Fetal Development*.)

Figure S-10.—Cervical Mucus Ferning



Just prior to ovulation, cervical mucus dries in a ferning pattern.

SOURCE: L. Speroff, R. H. Glass, and N. G. Kase, *Clinical Gynecologic Endocrinology, and Infertility*, (Baltimore: Williams & Wilkins Co., 1978), p. 433.

Cervical Mucus

The receptivity of cervical mucus to sperm is a critical determinant of female fertility:

- Cervical mucus quality varies in response to the hormonal shifts of the menstrual cycle, making a woman's mucus more receptive to sperm on some days than others. (See ***Ovarian Function: Indirect Indicators.***)
- Certain agents may stimulate a woman's production of sperm antibodies or change the consistency or pH of her cervical mucus, making her reproductive tract unreceptive to sperm.

Evaluating the compatibility of a woman's cervical mucus with her partner's semen is thus an important measure of a couple's fertility (13).

Sperm-Cervical Mucus Interaction

Tests of sperm-cervical mucus interaction are described earlier in this chapter (see Sperm ***Function***). Each examines the ability of the sperm to penetrate the mucus and sperm vitality after penetration (13,38)39). (See Tech. Note 10.)

Because of the fluctuations of both semen and cervical mucus quality, repetition of the test may be necessary to confirm results. Cross-testing of the fluids with control samples—i.e., testing the

male's semen against a standard cervical mucus sample and the female's cervical mucus against a standard semen sample—is also useful. (See Tech. Note 11.)

Limited sperm motility in cervical mucus may indicate the presence of antisperm antibodies, either within the donor semen (autoimmunity) or in the reproductive tract of the female. Screening for antisperm antibodies is particularly useful where sperm motility appears poor in cervical mucus while semen analysis results are normal, (See Tech. Note 2 and Tech. Note 12.)

Endometrial Cells

The capacity of the endometrial cells (cells lining the uterus) to respond to monthly hormonal secretions is an important component of female fertility. Cyclic changes in hormone levels stimulate endometrial cell proliferation each month, preparing the uterus for pregnancy.

Endometrial Biopsy

Appropriate endometrial growth is verified through microscopic observation of endometrial tissue. Tissue is extracted by endometrial biopsy, a procedure described earlier, (See ***Tests of Ovarian Function***, and Tech. Note 19.) observation of endometrial cells also permits diagnosis of endo-

metrial infection or disease (e.g., endometritis) that could impair fertility.

Tubal Patency/Uterine Structure

The passage of a fertilized egg through the fallopian tubes and its implantation in the uterine wall are necessary for the establishment of pregnancy. Verification of tubal patency and uterine structure are, therefore, important aspects of a female fertility evaluation.

Hysterosalpingogram

Imaging of the uterus and fallopian tubes is possible by injection of dye into the cervix and filming its spread through the peritoneal cavity. The procedure is safe and relatively painless. X-ray photography of the dye dispersion indicates any occlusion or convolution of the fallopian tubes that might prevent passage of a fertilized egg to the uterus. In addition, the size, shape, and position of the uterus and the presence of any abnormalities in the uterine wall are discernible with this technique (11,62,67).

Laparoscopy

If hysterosalpingography indicates normal tubal and uterine structure, laparoscopy may be useful. It affords the physician the opportunity for direct observation of the peritoneal cavity. The procedure is described in the discussion of direct measures of ovarian function (62).

Ultrasound

Imaging of the peritoneal cavity using ultrasound equipment may prove to be the preferred method of observation. The method is painless and, to date, does not appear to be detrimental in any way (12).

Implantation/Establishment of Pregnancy

There are no direct measures of gamete or zygote transport in humans. Consequently, the occurrence of pregnancy is the only indication that fertilization, transport, and implantation have been successfully achieved (45).

Human Chorionic Gonadotropin (hCG)

Human chorionic gonadotropin (hCG) is a substance that is secreted only during pregnancy. Blood and urine hCG assays are used to determine whether pregnancy has occurred (25,67):

- Presence of hCG in the blood serum is the earliest indication of pregnancy; i.e., it occurs before a woman misses her menstrual period (25).
- Home pregnancy detection kits screen for hCG in the urine. While the tests claim a high degree of accuracy and cost less than laboratory blood serum assays (\$10 as opposed to \$30), pregnancy cannot be detected as early in urine assays as it can when blood serum is used. *

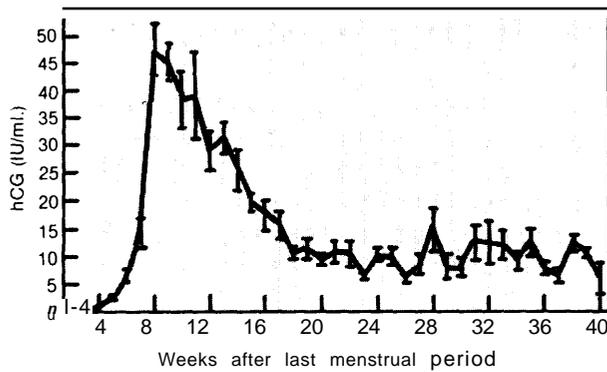
Because the amount of hCG in the blood follows a specific pattern over the course of the pregnancy (see figure 5-11), monitoring hCG is also useful in detecting pregnancy loss. Sudden drastic decreases in hCG indicate that pregnancy loss has occurred. (See Tech. Note 21.)

Embryonic Differentiation and Fetal Development

In-utero monitoring of embryo/fetal development is made possible by several clinical techniques, both invasive and noninvasive. Invasive procedures sample tissue and/or fluid in attempts to diagnose systemic diseases or disorders in the developing fetus. Noninvasive fetal monitoring covers a broad range of procedures, including several imaging techniques, designed to detect structural abnormalities and/or physical manifestations of disease in the fetus or in the maternal reproductive tract. Damage to the mother or conceptus may be the result of any number of factors (e.g., exposure to one or more reproductive hazards, injury, nutritional inadequacy). By affording prenatal diagnosis of damage and/or disease, these methods contribute information that may be critical to appropriate management of pregnancy.

*Human chorionic gonadotropin (hCG) is apparent in the blood serum about 15 days after conception (i.e., about the time the menstrual period is expected), while it is not apparent in the urine until 4 to 6 weeks after conception.

Figure 5.11.—Mean Human Chorionic Gonadotropin (hCG) During Pregnancy



SOURCE: A.C. Wentz, J.R. Givens, R.N. Anderson, et al., & Farrar/ of *Gynecologic Endocrinology and Infertility* (Baltimore: Williams & Wilkins Co., 1979).

Amniocentesis

The amniotic sac is the fluid-filled cavity that surrounds the developing fetus. (See figure 5-12.) Amniocentesis is the extraction of amniotic fluid for diagnostic purposes. * The fluid contains some live cells shed by the fetus. Both the fluid itself and the cells within it provide important information about the fetus.

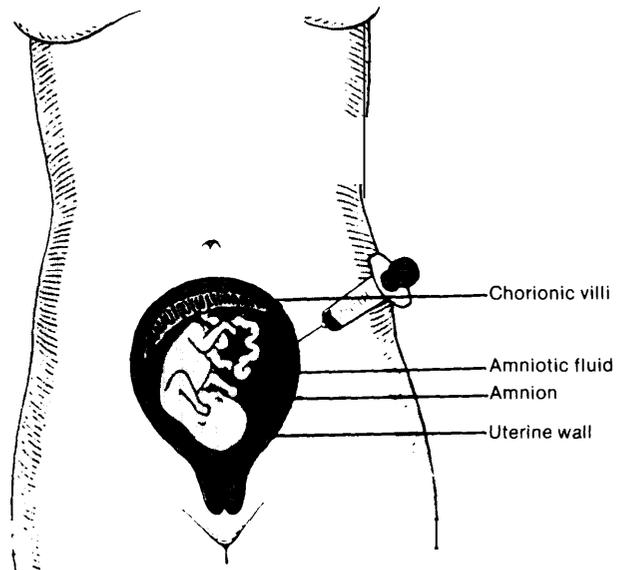
Amniotic cells are used primarily to diagnose chromosomal anomalies and genetic disorders:

- Disorders caused by aberrant chromosome structure or number (e.g., Down syndrome, Turner syndrome, Klinefelter syndrome) may be diagnosed by karyotyping* amniotic cells. (See Tech. Note 22.) Fetal sex is also apparent in the karyotyped cells.
- Several genetically based diseases—diseases caused by errors in the genetic information in a particular chromosome—(e.g., Tay Sachs, sickle-cell anemia, hemophilia) may be diagnosed using newly developed techniques. (See Tech. Note 23.) These are not routinely included in the analysis of amniotic cells, but are useful where specific genetic diseases are

*The fluid is extracted by means of a needle that is inserted through the abdomen into the amniotic cavity.

*● Karyotyping is a technique by which chromosomes are prepared for microscopic observation. It is a standard part of amniocentesis, [See Tech. Note 21.]

Figure 5-12.—Amniotic Cavity



Amniocentesis at 16 to 17 weeks.

SOURCE: Adapted from J T Queenan, "In Uterine Diagnosis of Down Syndrome," *Annals of NY Academy of Sciences* 171 :677, 1970.

likely (e.g., one or both parents suffer from a particular hereditary disorder). * * *

- Enzyme and protein assays of amniotic cells may identify certain other physiological disorders in the developing fetus. (See Tech. Note 24.) These assays are generally reserved for instances in which the presence of one of these disorders is suspected.

Amniotic fluid provides additional information about fetal health:

- The fluid is most commonly assayed for the substance alpha-fetoprotein (AFP). Abnormally high levels of AFP are associated with disorders of the central nervous system, particularly neural tube defects (e.g., anencephaly, spina bifida). Elevated AFP may also reflect other systemic disorders. (See Tech. Note 25.)

* * *Amniocentesis is considered a far safer diagnostic technique than those previously used to detect genetic disorders (e.g., fetal blood sampling to detect hemophilia, sickle-cell anemia, and other hereditary blood diseases).

- Hormone assays of the amniotic fluid are not a routine part of amniocentesis, but may be useful in diagnosing certain hormonal disorders in the fetus. (See Tech. Note 26.)

Amniocentesis is usually performed about mid-pregnancy (see Tech. Note 27) and is believed to involve a risk to the fetus of less than one percent (31). It has become relatively standard in the United States to offer the procedure to pregnant women over age 35* and to those at risk for certain fetal abnormalities. Table 5-2 outlines the situations for which amniocentesis is recommended.

Fetoscopy

The fetoscope is an optical instrument that allows direct observation of the fetus. Fetoscopy is an invasive procedure, like amniocentesis, but it presents a higher level of risk to both the pregnant woman and the fetus because the instrument

*Amniocentesis is advised for women who become pregnant during or after their mid-30s because there is an increased risk of Down syndrome associated with advanced maternal age.

Table 5-2.—Circumstances for Which Amniocentesis is Recommended

1. Pregnancies in women 35 years of age or older.
2. A previous pregnancy resulting in the birth of a chromosomally abnormal offspring.
3. Chromosomal abnormality in either parent, including:
 - a. balanced translocation^a carrier state
 - b. aneuploidy^b
 - c. mosaicism^c
4. Down syndrome or other chromosomal abnormality in a close family member.
5. Pregnancy after three or more spontaneous abortions.
6. A previous infant born with multiple major malformations on whom no cytogenetic study was performed.
7. Fetal sex determination in pregnancies at risk of a serious X-linked hereditary disorder.
8. Biochemical studies in pregnancies at risk of a serious autosomal or X-linked recessive disorder.
9. A previous child or a parent with a neural tube defect or routine screening finds maternal serum alpha-feto-protein level to be abnormally high.
10. Confirmation of certain abnormalities noted in a sonogram.

^aTh, shifting of a segment of one chromosome into another chromosome that does not result in any excess or lost genetic material.

^bAn, deviation from the correct number of chromosomes.

^cThe presence in an individual of two distinct cell lines for a single characteristic (e.g., two blood types).

SOURCE: Adapted from: J. A. Pritchard, P. C. MacDonald, and N. F. Grant, "Techniques to Evaluate Fetal Health," *Williams Obstetrics* (Norwalk, CT: Appleton-Century-Crofts, 1965), pp. 267-293.

is much larger and remains inserted for 15 to 45 minutes (42). The procedure is associated with a risk to the fetus of approximately 20 percent. Consequently, clinical use of this technique is extremely rare,

Nonetheless, fetoscopy can provide some information that amniocentesis and other diagnostic procedures cannot. Several congenital disorders that are not detectable through analysis of amniotic fluid and cells, for example, can be identified through fetoscopy, which allows direct sampling of fetal blood and/or tissue (24). Tissue samples may identify the presence of disease in the biopsied organ, while analysis of fetal blood may detect hemophilia or various hemoglobinopathies (deficiencies of the hemoglobin) (42).

The three uses for fetoscopy include:

1. viewing the fetus,
2. sampling fetal blood and/or tissue, and
3. in-utero therapy.

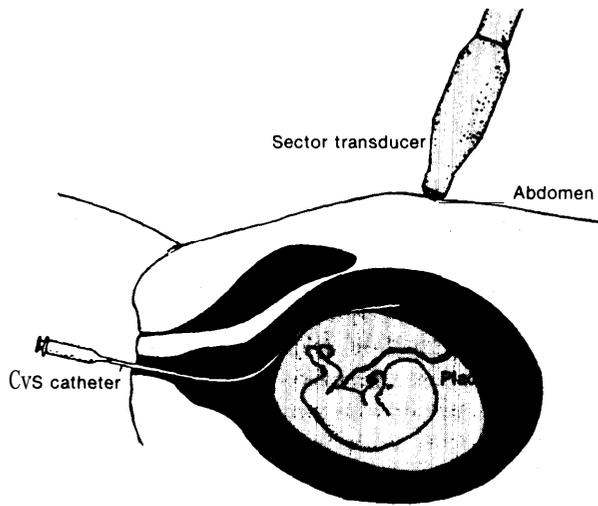
Because noninvasive imaging techniques (e.g., ultrasound) exist and appear to be safer, fetoscopy is rarely used where observation of the fetus is the sole aim. (See Tech. Note 28.)

Chorionic Villus Biopsy

Chorionic villus biopsy is a method of prenatal monitoring that permits early identification of various disorders, particularly genetically based diseases (e.g., hemophilia, sickle-cell anemia). The chorion (the membrane that encases the amniotic sac containing the developing fetus) is comprised of cells derived from, and thus genetically identical to, the fetal cells (57). (See figure 5-13.)

Analysis of chorionic tissue provides the same information as amniotic fluid and cells (53). The important advantage of chorionic sampling is that it can be done much earlier in pregnancy than amniocentesis or biopsy of other fetal tissues. Chorionic villus biopsy is, in fact, the only method for diagnosis of genetic disorders that can be performed in the first trimester of pregnancy. Both amniocentesis and fetoscopy require that the fetus be in at least the second trimester of gestation (57).

The degree of risk posed by the procedure is uncertain. Preliminary data indicate a high rate

Figure 5-13.—Chorionic Villus Biopsy

Diagrammatic illustration of the ultrasonically guided transcervical approach to chorionic villi sampling.

SOURCE: T. B. Perry, M. J. J. Vekemans, A. Lippman, et al., "Chorionic Villi Sampling: Clinical Experience, Immediate Complications and Patient Attitudes," *American Journal of Obstetrics and Gynecology* 151:161-166, 1985.

(12 percent) of fetal loss following chorionic villus biopsy (27). However, because the procedure is performed during the first trimester, during which time a high incidence of spontaneous abortion is normal, the post-biopsy losses may be attributable to normal early fetal loss rather than to the procedure itself (27)(57). Since the actual degree of risk associated with chorionic biopsy has not been established, those who choose to undergo the test must weigh the limited information about its dangers against the advantage of having a first-trimester prenatal diagnosis (54).

Ultrasonography

Ultrasound, described earlier in conjunction with assessment of ovarian and uterine activity, is an extremely useful method of analyzing embryo/fetal development. The procedure relies on differences in acoustic densities for information about the status of the uterus and its contents. To date, no adverse effects in humans have been found to be caused by ultrasonography (12). Consequently, it has largely replaced the use of X-ray in obstetrics (53).

Sonographic Imaging.—Over the course of pregnancy, ultrasound imaging affords a vast range of diagnostic possibilities:

- Early use of ultrasound can detect ectopic pregnancies and assess gestational age. (See Tech. Note 29.)
- Beginning the seventh week of gestation, ultrasound imaging enables the embryonal heartbeat to be "visualized."
- In the second trimester, ultrasound allows detection of gross fetal malformations (e.g., anencephalies), multiple pregnancies, placental localization, progression of fetal growth (26,31,55).
- In the late stages of pregnancy, ultrasonography is useful for monitoring fetal breathing, trunk and limb movement, filling and emptying of the bladder, and quantity of amniotic fluid (53).
- Ultrasound imaging equipment may be used in conjunction with other fetal diagnostic methods; e.g., to ensure proper placement of the needle in amniocentesis.
- Ultrasound imaging facilitates delivery of a fetus whose presenting part cannot be adequately determined during labor.

Table 5-3 provides a more detailed description of the range of uses for ultrasound in obstetrics.

Its safety and potential for identifying fetal abnormalities and for providing reassurance of fetal well-being make ultrasonography an attractive diagnostic technique. In parts of Western Europe and Scandinavia, ultrasonic surveillance is considered a standard component of obstetric care. The procedure is not, as yet, routine in the United States, however, partly because of its cost (approximately \$125).

Monitoring the Fetal Heart Rate.—[In addition to the use of diagnostic ultrasound, ultrasound equipment is routinely used to monitor fetal heart rate. (See Tech. Note 30.) Response of the fetal heart to uterine contractions and to fetal movement has been identified as an indication of fetal well-being. The hand-held ultrasound device is also used to monitor fetal heart rate during labor and delivery.

Table 5-3.-Use of Ultrasound in Fetal Monitoring

1. Very early identification of intrauterine pregnancy.
2. Determination of gestational age: permits proper timing and management of delivery.
3. Identification of multiple fetuses, including conjoined twins.
4. Demonstration of the size and the rate of growth of the amniotic sac and the embryo, and, at times, resorption or expulsion of the embryo.
5. Measurements of the fetal head, abdominal circumference, and femur (the bone that extends from the pelvis to the knee), to help identify the duration of gestation for the normal fetus or, when measured sequentially, to help identify the growth-retarded fetus.
6. Comparison of fetal head and chest or abdominal circumference to identify hydrocephaly (accumulation of fluid in the cranium), microcephaly (abnormal smallness of the head), or anencephaly (congenital absence of the cranial vault, with brain missing or drastically reduced in size).
7. Detection of fetal anomalies such as abnormal distention of the fetal bladder, ascites (accumulation of serum in the abdominal cavity), polycystic kidneys, renal agenesis (failure of kidney to form), ovarian cyst, intestinal obstruction, diaphragmatic hernia, meningocele (protrusion of brain membranes and part of the spinal cord through a defect in the vertebral column), or limb defects.
8. Demonstration of hydramnios (excess amniotic fluid), or oligohydramnios (inadequate levels of amniotic fluid) by comparing the size of the fetus to the amniotic fluid surrounding the fetus.
9. Identification of the location, size, and "maturity" of the placenta.
10. Demonstration of placental abnormalities such as hydatidiform mole (pregnancy abnormality resulting in a mass of cysts resembling a bunch of grapes), and anomalies such as chorioangioma (tumor of the chorion).
11. Identification of uterine tumors or anomalous development.
12. Detection of a foreign body such as an intrauterine device, blood clot, or retained placental fragment.
13. Monitoring fetal movement, including fetal heartbeat, breathing, trunk and limb movement, bladder function.
14. Adjunct to amniocentesis: guidance of the needle to avoid damage to placenta and/or fetus.
15. Adjunct to special procedures such as fetoscopy, intrauterine transfusion, and chorionic villus biopsy.
16. Follow-up observation of fetal anomaly identified by some other method; e.g., screening for anencephaly where amniocentesis indicates elevated alpha-f etoprotein levels.
17. Determination of fetal presentation to facilitate delivery, particularly when the presenting part cannot be adequately determined in labor or the fetal presentation is variable in late pregnancy.

SOURCE: Adapted from: J. A. Pritchard, P. C. MacDonald, and N. F. Grant, "Techniques to Evaluate Fetal Health," *Williams Obstetrics* (Norwalk, CT: Appleton-Century-Crofts, 1965), pp. 267-293; and Consensus Conference, "The Use of Diagnostic Ultrasound Imaging During Pregnancy," *Journal of the American Medical Association* 252:669-672, 1964.

x-ray Radiography

Use of diagnostic radiography in obstetrics has become limited for several reasons:

- Some evidence suggests a correlation between prenatal exposure to ionizing radiation and fetal defects (e.g., chromosomal damage, childhood cancer).
- Uncertainty regarding the effects of the radiopaque dyes used to enhance fetal imaging has raised concern (10). *
- Most of the measurements made radiographically (e.g., skeletal malformations, neural tube defects, gastrointestinal obstructions, fetal tumors) can also be made using ultrasound, a method for which no correlation with fetal damage has been identified (12,53).

Despite these concerns, limited use is still made of radiography in obstetrics, particularly in the third trimester of pregnancy, when evidence suggests the fetus may be least susceptible to radiologically induced defects (10,66). Pelvimetry (X-ray of the pelvic region), for example, may help to determine the need for cesarean section when a breech (bottom-first) presentation of the fetus is discovered during labor.

Nuclear Magnetic Resonance

Nuclear magnetic resonance is a method of organ and body imaging that may become an important obstetric tool once its safety during pregnancy can be established (53). Its utility for in utero observation of structure and function has already been documented (60,63).

Delivery and Lactation

Several toxic agents can affect the ease and timing of parturition. Techniques for monitoring the status of the fetus during labor and delivery,

*Visibility of the fetus is often enhanced through instillation of water-soluble (amniography) or lipid-soluble (fetography) dyes into the amniotic fluid that surrounds the fetus (10,40).

aimed at early identification and relief of fetal distress, may provide important insights into the impact of various exposures on these aspects of reproduction (45).

In most pregnancies, basic clinical monitoring of the fetal heart rate, frequency of uterine contractions, and rates of cervical dilation and descent of the fetus is adequate. The fetal heart rate is monitored using either a specialized stethoscope or a hand-held ultrasound device. The heart rate is measured either intermittently or continuously, with emphasis on the rate during and immediately following uterine contractions (53).

Continuous electronic monitoring of fetal heart rate and/or uterine pressure is indicated for certain conditions; e.g., maternal diabetes, previous unexplained stillbirth, induction of labor. The electronic equipment used for these procedures, however, requires invasion of the uterus and may pose some risk to the fetus (e.g., trauma, infection) (53).

Measuring fetal blood pH at regular intervals during labor and delivery also provides an indication of fetal well-being. Like electronic monitoring, however, it is reserved for specific instances because the taking of the sample may cause trauma, infection, or damage to the fetus (53).

Lactation

A woman's ability to produce and secrete milk may be adversely affected by certain toxic exposures. Competence of lactation is an important indicator of such damage (45). In addition, several substances have been found to contaminate the milk produced by women exposed to them. In such instances, chemical analysis of milk content may be necessary to verify its suitability for consumption.

Chemical Content of Milk. --Chemical analysis of milk content provides information on the presence of toxins that may pass to the infant during maternal feeding. Various chemical assay methods (e.g., gas chromatography and high pressure liquid chromatography) are available. Depending on the compounds involved, different techniques are appropriate (69). Procedure costs vary by several orders of magnitude (i.e., from \$5 to \$5,000) depending on the substance for which the screening is done. To date, chemical analysis of maternal milk is undertaken only when there is reason to believe that the milk source may be contaminated at levels sufficient to affect the nursing infant.

CONCLUSION

While there are several methods by which to estimate individual reproductive capacity, physical examination and laboratory analyses can only determine that a man or woman is **potentially** fertile. Fertility is a product of the specific interaction of a particular couple. Evaluation and treatment of infertility, therefore, must consider the couple as a unit.

Furthermore, a thorough assessment of reproductive capacity cannot be limited to an evaluation of reproductive organs and reproductive cells (sperm and eggs). The multitude of parameters that comprise reproductive health are inextricably related to other physiological systems. Physical examination of the fertility patient, for example, must include assessment of circulatory, endo-

crine, and neurologic function. Oral or written history-taking must consider a broad range of medical factors and lifestyle characteristics that may influence reproductive health. In conjunction with the appropriate laboratory analyses, these may contribute critical insights into the cause, diagnosis, and appropriate treatment of reproductive impairment.

Examination of the male fertility patient is simplified by the fact that his reproductive organs and germ cells (sperm) are readily accessible. The female correlates are not. However, while semen analysis does permit evaluation of several aspects of male reproductive function (e.g., ejaculatory capacity) and of semen quality and **quantity** there remains no positive method by which to dif-

ferentiate fertile and infertile sperm. Female reproductive health can be estimated through a variety of indirect indicators (e.g., menstrual regularity, hormone levels, cervical mucus properties) and direct methods (e.g., tissue biopsy, laparoscopy, ultrasound imaging). None of these, however, constitutes absolute evidence of a woman's ability to conceive or to maintain a pregnancy.

No diagnostic method, in fact, provides positive verification of individual reproductive capacity. Even techniques that consider the interaction and compatibility of a couple as a unit (e.g., sperm-cervical mucus interaction) cannot confirm their ability to generate healthy offspring. Successful

reproduction is the only absolute verification of a couple's reproductive potential.

The development of additional clinical methods may advance the evaluation of infertility and the in-utero diagnosis of fetal abnormalities, but monitoring their incidence in the population will continue to be important. Changes in frequency of reproductive difficulties (e.g., infertility, frequent miscarriage, premature birth, structurally and/or functionally impaired offspring) can provide insights into their causes, thus helping to identify those factors (i.e., workplace exposures, lifestyle characteristics) that impair human reproductive capacity.

TECHNICAL NOTES

1. Leutinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone are the hormones most frequently measured in the male. The proper balance of these substances in the bloodstream is critical to the entire range of reproductive functions. (See chapter 3.)

2. Antisperm antibodies in the male seminal fluid or blood serum (autoimmunity) can result in reduced fertility or infertility. Where sperm agglutination occurs with no evidence of bacterial infection, antibody testing may reveal autoimmunity. Various illnesses and surgical procedures (e.g., vasectomy, hernia repair, testicular infection, mumps, prostatitis) alert the physician to the possibility of antisperm antibodies. A simple test, combining semen, antibody-treated blood serum, and antibody compounds, detects the presence of antisperm antibodies in the semen (i.e., if it carries antisperm antibodies, sperm will adhere to the antibody-treated blood serum) (28). An alternative method uses *immunobeads*, compounds to which antisperm antibodies adhere. By suspending sperm in a solution of immunobeads, sperm to which antibodies have been bound are identifiable (9). (See Tech. Note 11.)

Antisperm antibodies can also occur in the female, causing impaired fertility. Where sperm of good quality show poor interaction with cervical mucus in the post-coital test, it is important to screen both the male and female for antisperm antibodies.

3. The first study to correlate sperm density with fertility cited 20 million sperm per milliliter of semen as the lower limit of a "normal sperm count." This finding was based on a comparison of sperm density in

1,000 fertile and 1,000 infertile men. The researcher noted that those identified as infertile frequently had sperm counts below the 20 million level (41).

Subsequent studies, however, demonstrate that pregnancy can occur even when the sperm density is well below that level. There remains no uniformly accepted specification of the number of sperm per milliliter of semen necessary to establish fertility (23,71).

4. Testicular biopsy is the surgical removal of a wedge of testicular tissue for analysis. Where normal spermatogenesis (sperm production) is occurring, microscopic observation of the tissue should disclose all stages of growth—from immature spermatocytes to mature sperm.

Lasography describes X-ray imaging of the ejaculatory tract following instillation of radio-opaque dye in order to locate any obstruction of the ejaculatory ducts (59).

5. In the visual rating system commonly used in laboratory motility assessment, semen is examined microscopically and the number of motile sperm in several areas of the microscopic slide is used to estimate the overall percentage of motile sperm in the semen. Individual sperm are often scored according to the following scale (23):

- 0 =No progression
- 1 =Weak forward progression
- 2 =Moderate forward progression
- 3 =Active forward progression

The extreme subjectivity of these ratings makes comparison of motility data from different laboratories problematic.

6. **Videomicrography.** Videomicrography is a recently developed technique that improves objectivity in assessing sperm motility. A video camera mounted on a microscope is used to record sperm activity. The distance traveled per second (swimming speed) by individual sperm is determined using a metered viewing screen. The percentage of motile sperm in the total sample can also be estimated by this method (37).

7. **Single Image Photomicrography/High Speed Cinemicrography.** Single image photography permits observation of sperm movement. Forwardly progressing sperm appear as streaks in the time-exposed photograph. (See figure 5-4.) The swimming speed of these cells is determined by the length of the "streak" in relation to the time of the photographic exposure (e.g., 25 micrometers per second) (29,43,50).

8. **Automated Analysis.** To further reduce human error and subjectivity in analysis of sperm movement, automated techniques have been introduced. These rely on computerized scanning and evaluation of photographic images to determine individual sperm velocities and the percentage of motile sperm in a sample (2,34).

9. Reference slides (70) and morphology overlays (36) are two recently developed methods that attempt to standardize morphology assessment. These establish categories (e.g., narrow head, large head, pear-shaped head) and provide standards against which to measure sperm (36,70). (See figure 5-7.)

An alternative approach is to perform direct morphometric measurement (i.e., length, width, area, circumference) of at least 50 sperm from a sample. Initial studies suggest that morphologic consistency of the sperm in a sample may correlate with fertility (35,58).

10. In the **post-coital test**, sperm are observed in a cervical mucus sample taken shortly after intercourse. An alternative method obtains separate samples of semen and cervical mucus and combines them in the laboratory to observe their interaction. Results of the post-coital sperm-cervical mucus interaction test do correlate with fertility (21).

11. The semen of the male partner can be tested against a standardized cervical mucus sample (e.g., bovine and synthetic mucus are under study); while the woman's cervical mucus can be tested for its receptivity to a semen sample of good quality. This enables the physician to determine whether the couple's fertility problem is attributable to one of the two partners or is the result of a compatibility problem.

Semen: male patient	X	Cervical mucus: female patient
Semen: control specimen	X	Cervical mucus: control specimen

12. Studies indicate that antisperm antibodies can be categorized according to their binding point on sperm. Those that bind to the head region appear to be most obstructive to sperm penetration of cervical mucus and/or zona pellucida of the egg cell, while tail-binding antibodies may impair sperm motility. One recently developed method enables the site of sperm-antibody binding to be identified. The technique uses immunobeads, compounds that adhere to antisperm antibodies. By suspending sperm in a solution of immunobeads, sperm to which antibodies have been bound are identifiable (9).

13. The **zona-free hamster egg penetration test** examines the ability of human sperm to penetrate hamster eggs from which the outer layer—the zona pellucida—has been removed. The zona pellucida is the major barrier to fertilization between animals of different species (1).

A substantial weakness of the testis that sperm that are able to penetrate a zona-free hamster egg may be unable to fertilize a human egg with its zona pellucida intact. The result is that men who have demonstrated fertility problems may appear normal in the zona-free hamster egg test. Studies note the occurrence of such "false positive" results (51). The test may also show "false negative" results, indicating infertility in males who have recently fathered children (19).

Studies show that penetration rates of sperm from fertile men range from 11 percent to 100 percent in sperm-egg penetration tests (1). Researchers disagree as to what constitutes "normal sperm penetration." Some identify a male as fertile if 90 percent of his sperm successfully penetrate the test egg, while others consider a single penetration an indication of reproductive competence (19).

14. There are exceptional cases, such as the event of an **anovulatory** cycle—menstruation occurring without an egg passing to the uterus. In most instances, however, regularity of menstruation is an indicator of reproductive health.

15. Assay of salivary fluids for progesterone has been suggested as an alternative to blood serum assays for this hormone. The method is particularly *advantageous* where serial sampling is required to monitor daily fluctuations in progesterone levels. Because adequate luteal function is reflected by ovarian secre-

tion of progesterone, the technique may also be useful in identifying luteal phase deficiency (74).

16. The increase in progesterone production that follows ovulation causes a rise of 0.5 to 1.0° in basal body temperature. These temperature shifts can be measured and recorded by the woman herself with a standard oral or rectal thermometer. Because the basal temperature reflects the lowest or resting temperature, she must take the reading immediately on waking in the morning, before arising from bed (56,67).

Although problems with the reliability of this method have been identified, many laboratories believe basal body temperature to be an extremely sensitive and accurate indicator of ovulation. They base the timing of subsequent fertility assays on the occurrence of these temperature shifts.

17. The large quantity of estrogen present immediately before ovulation stimulates increased production of cervical mucus (from 20 to 60 mg/day to 200 to 700 mg/day) (73). This mucus has particular characteristics that identify it as "preovulatory mucus." It is more watery, less viscous, and displays a "fern" drying pattern due to the crystallization of salt on the mucus filaments (see figure 5-10) (6,56,67).

18. A typical luteal phase is precisely 14 days long. Variation among women in length of menstrual cycles is usually due to differences in the number of days **preceding** ovulation while the luteal phase remains 14 days in most women. Deviation indicates a luteal phase deficiency (56,67).

19. In endometrial biopsy, uterine tissue samples are obtained by scraping the uterine wall with a small instrument inserted in the endocervical canal. Microscopic observation of the endometrial cells verifies cell proliferation in response to monthly hormonal secretions.

The degree of endometrial cell development indicates a woman's menstrual stage and allows the date of her next menses to be predicted. For example, if the endometrial tissue obtained in the biopsy shows development characteristic of the **22nd day** of the cycle, menstruation should occur 6 days later (i.e., on the **28th day**). If menses occurs sooner or later than this expected date, a luteal phase deficiency is identified (56,67).

20. The tissue samples taken in a laparoscopic ovarian biopsy represent only a minute area (0.5 centimeters) of the ovary. Other regions of the organ may vary considerably. Thus, even ovarian biopsy cannot provide a complete image of the ovary and the number of oocytes it contains.

21. Identification of pregnancy through hCG monitoring is most useful in the case of early pregnancy losses, which are otherwise difficult to detect (i.e., loss before pregnancy is visibly apparent). Some findings indicate that as many as 70 percent of all pregnancies are lost before the pregnancy itself is recognized. This is due, in part, to the amount of time that may elapse before a woman realizes that she is pregnant. Because hCG monitoring provides earlier indication of pregnancy, it could prove useful in establishing more accurate estimates of early pregnancy loss rates (16,25).

22. In karyotyped amniotic cells, numerical aberrations (more or less than the standard 46 chromosomes) as well as structural abnormalities (deleted or misplaced regions of the chromosomes) that result in abnormal formations (e.g., rings, fragments, chromosomes with obvious lesions) are detected (31). Several human disorders (e.g., Down syndrome, Turner syndrome, and Klinefelter syndrome) are known to result from these chromosomal anomalies.

23. Recently developed techniques enable a number of genetically based diseases (i.e., diseases caused by errors in the genetic information in a particular chromosome) to be diagnosed using amniotic cell chromosomes. The most common of these is a genetic mapping technique that uses enzymes (restriction endonucleases) known to cleave DNA in specific code locations. Chromosomes bearing properly coded genes yield a particular pattern of fragments when cleaved by the enzymes, while chromosomes with alternate forms of these genes are cleaved differently (14,47,48). Some diseases that are the result of a faulty gene (e.g., Tay Sachs, sickle cell anemia, hemophilia) are identifiable with this method.

24. Enzyme and protein assays of amniotic cells are another means of diagnosing certain disorders in the developing fetus. Presence of one protein (the glial protein S-100), for example, indicates the likelihood of a central nervous system defect, while enzyme assays can detect certain metabolic disorders, such as the inability to digest specific amino acids, lipids, or sugars (31). These assays are generally reserved for instances in which the presence of one of these disorders is suspected.

25. Alpha-fetoprotein (AFP) is a protein synthesized by the fetus and present in the amniotic fluid in concentrations that decrease with gestational age. Determination of AFP levels is a standard part of amniocentesis. Abnormally high levels of AFP are associated with disorders of the central nervous system, particularly with neural tube defects (e.g., anencephaly, spina

bifida). Elevated AFP levels (greater than 20 milligrams per milliliter) may reflect other disorders, such as atresias (abnormal closures) of the digestive tube, polycystic kidneys, annular (ringlike) pancreas, hydrocephalus (accumulation of fluid in the cranium), and Fallot's tetralogy (congenital cardiac defects).

26. Proper gonadal development in the fetus requires the appropriate balance of gonadotropins and steroid hormones. The levels of these substances may be determined by analyzing the amniotic fluid (31).

27. Amniocentesis performed earlier than the 16th week often fails because of difficulties in obtaining an adequate amount of amniotic fluid and in successfully culturing the amniotic cells during the first trimester of pregnancy (24).

28. Uses of fetoscopy include:

- Viewing the Fetus: The small lens of the fetoscope allows detailed observation of approximately 2 to 4 square centimeters of the fetus at one time (42). This facilitates prenatal diagnosis of major external morphological malformations including facial clefts, deformed ears, limbs, and genitalia. Because noninvasive imaging techniques (e.g., ultrasound) exist and appear to be safer, fetoscopy is rarely used where observation of the fetus is the sole aim. The limited size of the fetoscope field prevents visualization of the fetus as a whole. It cannot be used to assess such things as limb size, thoracic volume, and overall anatomical symmetry (42).

- **Sampling Fetal Tissue:** The most substantial benefit that fetoscopy provides is that it permits access to fetal blood and tissue (24). Samples of the blood, skin, and/or liver tissue are taken with the fetoscope in place. Tissue samples may identify the presence of disease in the biopsied organ, while analysis of fetal blood may detect hemophilia or various hemoglobinopathies (deficiencies of the hemoglobin) (42). Further development of fetal blood assays may permit prenatal diagnosis of enzyme deficiencies, nutritional and metabolic disorders, and blood cell diseases (24).

- **Therapeutic Uses:** Development of therapeutic uses of fetoscopy, such as blood transfusions to immunodeficient fetuses, may make it a valuable method for early diagnosis and correction of fetal disorders (24). Present use of fetoscopy, however, remains limited by the level of risk posed to the developing fetus (42).

29. A delivery that is too early or too late may jeopardize the fetus. Accurate estimations of gestational age made with ultrasound are useful in determining proper timing and management of delivery (i.e., in determining the need to suppress or to induce labor).

30. In the **Contraction Stress Test**, uterine contractions are stimulated (e.g., by injection of oxytocin) and the fetal heart response monitored with ultrasound equipment. The **Nonstress Test** uses ultrasound to reflect the fetal heart response to fetal movement as identified by the mother (53).

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