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FEMALE REPRODUCTIVE DISORDERS

Workgroup Members

Anthony R. Scialli, MD, Cochairperson; Shanna H. Swan, PhD, Cochairperson; Donna D. Baird, PhD; Brenda Eskenazi, PhD; Ginger Gist, PhD; Maureen C. Hatch, PhD; James S. Kesner, PhD; Grace K. Lemasters, PhD; Michele Marcus, PhD; Maureen E. Paul, MD; Paul Schulte, PhD; Zachary Taylor, MD; Allen J. Wilcox, MD, PhD; Chris Zahniser, RN, MPH.

Summary: *Members of the workgroup on female reproductive disorders discussed methods to evaluate five principal functions: menstrual dysfunction, infertility, pregnancy loss, pregnancy complications, and lactation disorders. To test each function, a nested strategy was considered, based on three progressive levels of effort (highest, medium, and lowest) available to conduct field investigations. This strategy was analogous to the three-tier classification of biomarkers used by other workshops. The lowest level of effort, corresponding to Tier 1, consists only of questionnaires, diaries, and reviews of maternal and infant medical records. The medium level of effort (Tier 2) adds some laboratory analyses, for example, measurement of progesterone*

in saliva and several glycoprotein hormones in urine to evaluate menstrual dysfunction, infertility, and pregnancy loss. The highest level of effort (Tier 3) involves prospective collection of diary information and simultaneous collection of biological specimens.

INTRODUCTION

ADVERSE REPRODUCTIVE OUTCOMES

Assessment of reproductive function in women is complicated by the cyclicity of this function; by the inaccessibility of the germ cell, gonad, and zygote; by the multiplicity of reproductive roles served by women (ovum production, ovulation, environment for fertilization, environment for gestation, and lactation); and by the variability in reproductive parameters within and between women. Evaluation of possible reproductive dysfunction is most effectively directed to the specific adverse reproductive outcome for which there is a suspected abnormality. The workgroup divided the discussion of female reproductive function into the following five adverse outcomes, each of which is discussed in this chapter:

1. Menstrual dysfunction.

2. Infertility.

3. Pregnancy loss.

4. Pregnancy complications.

5. Lactation disorders.

The methods described herein are designed to evaluate the effects of exposures on the population, not the individual. A modest reduction in a given parameter (for example, luteal phase

progesterone production) might have negligible effects on the reproductive potential of most women, but might render infertile women who make up a marginally fecund segment of the population.

In the evaluation of a population for which there are concerns about female reproductive disorders, selection of the adverse outcome(s) of interest is made based on individual factors in the community. Perhaps the most compelling of these factors is the population size available, because sample size is an important determinant of study power and feasibility. Studies of menstrual function can involve women of reproductive age who are ***not*** using hormonal contraception and can permit larger samples in a given population than can infertility studies (which evaluate only sexually active women who are not using—or irregularly using—contraceptives) or pregnancy loss studies (which evaluate only pregnant women). Another consideration involved in outcome selection is the health concerns of the community, provided the community reports of cases have been confirmed. Because exposures can affect multiple end points, a study might attempt to address all of these concerns.

It should also be recognized that toxicologic information about the exposures of concern might be very useful in guiding the choice of adverse reproductive outcome(s). Animal studies can provide important clues about the potential reproductive effects of toxicants. Information might also be available about related compounds. That is, an exposure involving an organochlorine pesticide might be evaluated for an association with menstrual dysfunction based on the estrogenicity of many members of this class of compounds.

LEVELS OF EFFORT

In selecting those adverse reproductive outcomes to be evaluated, one must recognize that different levels of time,

energy, and money might be available for an evaluation effort. The quality of the data obtained in a study can be expected to depend directly on the level of effort invested in the study. All levels of effort described in this chapter involve a baseline questionnaire to collect information on menstrual and reproductive history and potential confounding or covarying factors (discussed in the following sections). The highest level of effort (corresponding to Tier 3 biomarkers) for female adverse reproductive outcomes involves prospective acquisition of diary information with confirmation by biologic sampling. The medium level of effort (corresponding to Tier 2) drops some or all of the biologic sampling but retains the prospective information collection. The lowest level of effort (corresponding to Tier 1) drops the prospective elements and adopts the baseline questionnaire as the instrument in a cross-sectional design. This lowest level could be supplemented by prospectively acquired information in a subset of study participants for validation of the instrument. This nested strategy is illustrated in Table 2-1 for three of the adverse reproductive outcomes.

The choice of level of effort is guided by several considerations, including exposure assessment. It makes little sense to mount an elaborate, expensive study unless an exposed population can be identified. Sample size and cost are also important considerations. The use of daily urinary human chorionic gonadotropins (hCG) to identify very early pregnancy loss requires a sufficient number of sexually active women who are not using contraceptives, and is very expensive. Use by study participants of less expensive home kits for hCG at designated times of the cycle (for example, the first 2 days of menstruation) will miss some transient hCG rises, but will still identify most early pregnancy losses. This is a practical compromise that should give acceptable information in evaluating community concern about miscarriages (spontaneous abortions).

Table 2-1.—Levels of effort in reproductive studies: menstrual dysfunction, infertility, and pregnancy loss.

Measure	Level of Effort		
	Highest	Medium	Lowest
Baseline questionnaire	✓	✓	✓
Daily diary	✓	✓	
Saliva progesterone	✓*	H	
Urine human chorionic gonadotropin (hCG)	✓	H	
Urine luteinizing hormone (LH)	✓	H	
Urine follicle stimulating hormone (FSH)	✓		
Urine estrone 3-glucuronide ($E_1$3G)	✓		
Urine pregnanediol 3-glucuronide (Pd3G)	✓		

✓-Present in the protocol as a daily collection.
H-Simple home test or home collection methods are available and should be considered to add to the quality of the study; can be performed during a targeted part of the menstrual cycle rather than daily.
*Unless urine Pd3G is measured.

BASELINE QUESTIONNAIRE

A questionnaire designed to evaluate reproductive and menstrual cycle experience is an important component of all types of studies of female reproductive disorders. This

instrument can be used to collect information on potential confounding or covarying factors (Table 2-2). Often, questions on reproductive and menstrual function are incorporated in general health assessment instruments that are used in communities with health concerns. These questions might serve a Tier 1 function in directing attention to the need for more complete evaluation of one or more of the five female reproductive disorders. However, the baseline questionnaire used in reproductive studies is a detailed instrument that must be piloted in the population in which it is to be used.

MENSTRUAL DYSFUNCTION

GENERAL CONSIDERATIONS

Infertility is the adverse health outcome most often considered in relation to menstrual dysfunction. However, the presence of hormonal disturbances, reflected in menstrual parameters such as anovulation, short luteal phase, and progestational insufficiency, can also affect the rate and severity of health outcomes other than reproductive disorders, including osteoporosis, breast cancer, and cardiovascular disease.

Multiple parameters can be used to assess menstrual function. Using biologic monitoring, each cycle can be classified according to the presence or absence of ovulation and described by continuous variables, such as the length of the luteal phase or the magnitude of the post-ovulatory progesterone rise. When hormone measurements are available for multiple cycles per woman, the woman's menstrual function can be summarized across cycles using both means (or medians) and within-woman variability.

Studies of menstrual function are not limited by the severe sample size restrictions that limit other studies of female reproductive disorders. The National Survey of Family Growth

Table 2-2.—Potential confounding or covariate factors that might be collected in baseline questionnaires.*

Reproductive Factors	**Current-Pregnancy Factors**
Age at menarche and menopause	Gestational age at pregnancy awareness
Last menstrual period	Desire for pregnancy
Characteristics of menstruation	Presence of nausea
Regularity	History of febrile, viral, or other illness
Amount and duration of flow	
Associated pain	**Demographic Factors**
Parity	Date of birth
Term births	Race or ethnicity
Preterm births	Socioeconomic status
Stillbirths	
History of infertility	**Psychosocial Factors**
History of miscarriage	Tobacco use
Induced termination (including reason)	Alcohol use
Genital tract surgery	Drug use
Uterine anomalies	Stress
Polyps, hyperplasia	Exercise level and pattern
Fibroids	Occupational exposures
Endometriosis	Environmental exposures
Pelvic infection	
Polycystic ovarian syndrome	**Medical Factors**
	Medication or drug use
Sexual Factors	Therapeutic irradiation
Contraception	In utero DES
Frequency of intercourse	Genetic conditions
Use of lubricants	Systemic diseases
Number of partners	Galactorrhea
	Male factors

*Not all variables are appropriate in every study.
DES—Diethylstilbestrol.

estimates that only 10% of women of reproductive age become pregnant each year, and this number limits the population available for the study of pregnancy-related outcomes. Menstrual

function, on the other hand, can be assessed in all women of reproductive age who have intact ovaries and who are not taking hormonal medication or contraceptives. In fact, biologic specimens obtained from fewer than 100 women can provide meaningful data on menstrual function, particularly if one or more specimens are obtained from each woman during several of her menstrual cycles.

HIGHEST LEVEL OF EFFORT

The methods suggested for the most complete assessment of menstrual function (baseline questionnaire, daily diary, and urine collection) are also appropriate for studies of infertility and pregnancy loss (discussed in a later section).

The highest level of effort for evaluating menstrual function is extremely intensive and costly. As outlined in Table 2-1, this level of effort includes the use of a baseline questionnaire to obtain a retrospective history, a diary to obtain prospective information on exposure and confounders, and daily urine collection for assessment of steroids and gonadotropins. The initial studies conducted using these methods were invaluable in establishing protocols and baseline values. In the future, however, these methods should be used to evaluate risks to exposed populations only when there is a clear demonstration of exposure to an agent with a high likelihood of reproductive toxicity.

Baseline Questionnaire

The baseline questionnaire should include many or most of the basic demographic and exposure variables that are germane to female adverse reproductive outcomes (Table 2-2). For assessing menstrual function, this questionnaire should include usual cycle length, amount of bleeding, intermenstrual bleeding and symptoms, painful menses, symptoms of premenstrual syndrome

(PMS), and menopause. Recent use of oral contraceptives, date of termination of last pregnancy, and current or recent lactation should also be noted.

Daily Diaries

A prospective menstrual calendar provides information on timing and amount of bleeding with a precision not otherwise available. While obtaining the menstrual calendar is relatively inexpensive, identifying a cooperative population might require extensive screening. Furthermore, while only a single contact is required (the calendar can be mailed back), experience has shown that collection for more than one cycle is improved by reminder calls or field visits (which occur naturally if urine is also collected). Calendar items include medication use (such as oral contraceptives and other hormones), bleeding or spotting, and indication of heaviness of menstrual blood flow. Symptoms of PMS (headache, bloating, hours slept, cramping, and some psychological measure appropriate to assessing PMS), symptoms of pregnancy, and other symptoms might also be included. The daily diary provides an opportunity to collect serial data on potentially confounding variables such as active and passive exposure to cigarette smoke; consumption of alcohol and caffeine; exercise; weight; and psychosocial and physical stressors, including major life events such as divorce or death of a family member. Sexual activity and contraception, which are usually included in calendars designed to assess infertility and pregnancy loss, might also be useful in the interpretation of menstrual variation. However, this information is not essential to assess menstrual function, and should be requested with discretion.

These diaries and questionnaires have been used in several field settings, and response and cooperation have varied with population characteristics (such as socioeconomic status and ethnicity). This suggests that work is needed to better understand

how these instruments are interpreted by demographically diverse populations.

Assessment of Steroids and Gonadotropins

The most complete menstrual function study will include specimen collection in order to assay daily levels of steroids and gonadotropins. Serum assays are not recommended because of their invasive nature, the requirement for trained personnel, and the need for study participants to appear at the clinic daily. Urine specimens, on the other hand, can be collected easily by the study participant in her own home. Urine assays might actually be preferable to serum because they integrate pulsatile signals, although levels can fluctuate due to metabolic variation. Urine assays might benefit from creatinine correction to adjust for differences in urine concentration.

Based on recent urine collection studies, it is recommended that the first morning voided specimen be collected daily for one to six cycles (with other collection times noted on the menstrual calendar). Only a small amount of urine, ≤ 5 milliliters (mL) is required for most laboratories. These small vials can be packed in waterproof cardboard boxes (32 per box) with self-contained calendars that aid a woman in keeping specimens in calendar order. Several of these boxes can be stored in most home refrigerators or freezers with little difficulty. Freezing is not required. Some investigators recommend that a small amount of glycerol be added to prevent degradation (induced by freeze-thaw) of specimens that will be assayed for gonadotropins (*1*).

Sample size will be limited by the size of the exposed population and by the resources available for recruitment, participant contacts, field work, and bioassay procedures. The cost per assay might be high (more than $5 per specimen), but laboratory personnel costs can be reduced by employing a computerized specimen tracking system, particularly useful for large sample sizes.

Specific Assays

The urinary metabolites usually measured to assess menstrual function are pregnanediol 3-glucuronide (Pd3G), estrone 3-glucuronide ($E_1$3G), luteinizing hormone (LH), and follicle stimulating hormone (FSH). Current laboratory methods for measuring hormones are gas chromatography and immunoassays that use radioactive, enzyme, or fluorescent labels. The immunoassays can be competitive or two-site noncompetitive "sandwich" configurations. The performance of many of these immunoassays across laboratories has been compared and considerable variation has been observed. Even the same reagents when used in different laboratories can yield quite varied results. This finding underscores the importance of carefully evaluating the assay and laboratory to be used and the value of interlaboratory comparability for nonstandard assays. It also underscores the importance of using the same assay and laboratory to study the exposed and unexposed populations being compared.

Pregnanediol 3-glucuronide.—Pregnanediol 3-glucuronide (Pd3G) is used to evaluate the function of the corpus luteum, indicating that ovulation has occurred. Since the level of excretion of Pd3G is a function of metabolism, correcting or normalizing luteal phase Pd3G to baseline follicular phase levels might provide a more useful parameter than absolute Pd3G (*2,3*). Cycles for which no rise is observed can be denoted as anovulatory. However, cycles in which the rise is intermediate might reflect ovulation in the presence of a high baseline background Pd3G or abnormal corpora luteal function. Until this is better understood it might be useful to analyze such questionable cycles separately. Thus, each cycle can be classified as ovulatory, anovulatory, or questionable.

For cycles classified as ovulatory, the day of ovulation or luteal transition can be determined by plotting the ratio of $E_1$3G to Pd3G for each cycle day (*4*). Use of this ratio has the

advantage that no correction for creatinine is required and variation due to metabolism is minimized. The urinary LH surge might help identify this day in some cases.

Estrone 3-glucuronide.—Estrone 3-glucuronide ($E_1$3G) is used together with Pd3G in determining the timing of ovulation and luteal phase length. In addition, the height of the $E_1$3G peak and the area under the $E_1$3G curve during the follicular phase can provide information on follicular growth.

Luteinizing Hormone.—Luteinizing hormone (LH) provides the most precise endocrine measure of ovulation, when detectable. The transient nature of the LH surge can render it undetectable, although this can vary with the immunoassay (*5,6*). Freezing of specimens without the addition of glycerol can cause loss of LH activity (*1*). Use of an assay that captures both LH and LH-alpha might avoid this problem (*2,3*). Elevated LH levels in the presence of normal FSH levels can signal menstrual dysfunction such as polycystic ovarian syndrome (*7*).

Follicle Stimulating Hormone.—Follicle stimulating hormone (FSH) is the least frequently assessed of these four female reproductive hormones. FSH increases with age and increased FSH can reflect refractoriness of follicles or ovarian failure. In addition, high follicular phase FSH has been associated with reduced fertility (*8-12*).

Medium Level of Effort

It is also possible to employ the baseline questionnaire and calendar with biologic markers of hormone function that are less sensitive or specific than urine assays. One alternative is salivary progesterone. Salivary estrogen levels are too low for detection by most immunoassays and the glycoprotein hormones LH, FSH, and hCG are not present in saliva. Because only progesterone unbound to blood proteins passes into the saliva, this affords an

index of biologically active progesterone. Progesterone measured in this way is not subject to metabolic variation but is subject to variation due to pulsatile release from the corpus luteum. The resultant variability in Pd3G and its effects on sample size requirements are currently unknown.

Saliva specimens are easy to collect and stable at ambient temperature for up to 6 months, although longer storage might require the addition of a bacteriostatic agent (*13*). Because protein-bound steroids in blood-contaminated saliva can falsely elevate progesterone levels, there is an advantage to using an osmotic device to generate an ultrafiltrate. One such device for passive collection is the SalivaSac™. The disadvantages of this device include limited specimen volume (1 to 1.5 mL) and possible immunoassay interference by the osmotic substrate (sucrose).

Alternatively, ovulation can be more crudely evaluated using a commercially available semiquantitative hormone kit. There are several available for identifying the preovulatory LH surge. These cost about $35 for six determinations. The sensitivity of commercial kits in predicting ovulation varies among different commercially available products and can range from 55% to 100% (*14-16*). Kits for detecting Pd3G in urine in the luteal phase to determine whether ovulation has occurred are available for research purposes (Monoclonal Antibodies, Inc.), but not yet commercially available. Using semiquantitative hormone kits has the advantage of eliminating the cost of urine or saliva collection and storage, though not the costs of participant recruitment and followup. However, errors in reporting results can be high.

LOWEST LEVEL OF EFFORT

It is also possible to use only the baseline questionnaire and daily diaries when biomarkers are not warranted or feasible. Recruitment should be somewhat more successful if participants are not expected to collect biologic specimens, and this design

will be less costly. If accurately maintained, menstrual diaries will reflect all episodes of bleeding, including breakthrough bleeding. However, the measures of menstruation obtained in this way will be insensitive and nonspecific compared to hormone data; that is, apparently normal intermenstrual intervals might mask abnormal ovulatory or even anovulatory cycles.

Daily basal body temperature can also be used to provide some information on the timing of ovulation, although these measures also have limited sensitivity and specificity (*17,18*). Recording the quality of cervical mucus can provide a rough marker of cycle day in ovulatory cycles. However, there is considerable loss in the amount and quality of information with these methods when compared with urine collection methods (*16,19*).

A simple retrospective questionnaire can provide some information on menstrual function in a large sample, although retrospective reporting of menstrual function might not correlate well with prospectively collected data.

INFERTILITY

GENERAL CONSIDERATIONS

This section discusses infertility, fecundability, and time to conception, all of which can be used to measure fertility potential. Infertility is defined as failure of a couple to conceive after 12 months of unprotected coitus. Couples attempting to achieve pregnancy are more likely to conceive than those who are not, even though both groups might not be using contraceptive methods. Couples attempting to conceive might also be less fertile than those that have already conceived. A study population might contain a large proportion of couples who use contraception irregularly; these couples cannot be considered

infertile, regardless of the number of months of irregularly used contraception. Finally, it should be recognized that a population of couples attempting to conceive might be less fertile than those who have already experienced conception.

Fecundability is the probability of a pregnancy in a noncontracepting cycle. Fecundability is about 20% to 25% for normal couples in the first cycle of attempting pregnancy. As more fertile couples become pregnant and drop out of the population at risk for pregnancy, the fecundability of the population decreases. After 12 months of unprotected coitus, about 80% to 85% of couples will have achieved a pregnancy.

Fecundability assessment can be refined by methods that detect the proximity of coitus to ovulation. If a couple do not have coitus within about a week prior to ovulation up to perhaps a day after ovulation, pregnancy in that cycle is unlikely.

One measure of fertility is time to pregnancy. This is a measure of the number of noncontraceptive cycles required for conception. In one study, 72% of couples had conceived by the fourth month (*20*).

Highest Level of Effort

Prospective evaluation of time to pregnancy is usually made in studies of couples who are not using contraceptives. Fecundability is often measured in such couples, as well as in couples who are using contraceptives irregularly. These methods involve use of menstrual diaries (see Menstrual Dysfunction), which include an indication of timing of coitus and use of contraception. These diaries can be supplemented with daily urine collection for determination of steroids ($E_1$3G and Pd3G) and pituitary gonadotropins (FSH and LH). Measurement of urinary hCG can be included for a uniform early diagnosis of pregnancy. When urine is not available, salivary progesterone

can be used. As described previously, for ovulatory cycles the day of luteal transition, characterized by a shift from a high to a low ratio of $E_1$3G to Pd3G, is taken as an indicator of ovulation.

About 20% of reproductive-age women in a population will be eligible and willing to participate in such a study. Women who use contraception can still be eligible to participate if the investigators exclude from analysis any cycles (over a time period that includes the day of ovulation) in which contraception was used. It might be more efficient to exclude women who definitely do not want to become pregnant, whether or not they use contraception, because these women are often successful in avoiding pregnancy. In the enrollment process, a baseline questionnaire is used to obtain eligibility information and information on potential confounders and covariates (Table 2-2).

Medium Level of Effort

Prospective collection of information by diary can be used without obtaining the daily biologic specimens. This design can be improved by using biologic sampling performed by the woman at home, eliminating the logistic and cost factors of storing and shipping specimens. These methods are discussed in a previous section (Menstrual Dysfunction).

Lowest Level of Effort

A retrospective questionnaire can be used to obtain information about past menstrual cycles. In many respects, this will be similar to the baseline questionnaire used in the enrollment of women for studies designed for greater levels of effort. A question such as, "Have you ever taken more than 12 months to get pregnant?" can be used to assess fertility and subfertility. In addition, women who are not using contraception can be asked, "How long have you been sexually active without

contraception?" Time-to-pregnancy data can be collected for planned pregnancies. This information has limitations. Women who have never been pregnant might represent the most adversely affected subgroup in a population exposed to a reproductive toxicant, and their experience will not appear in retrospective time-to-pregnancy data. Women with unplanned pregnancies, who might represent a more fertile subgroup, will also not be represented. Even without these potentially important subgroups, a shift in the distribution of time to pregnancy of a population might be detected, leading to recognition of an adverse effect on fertility.

There are important cultural differences in how women respond to questions about pregnancy and sex. For example, whether a woman reports a past elective termination of pregnancy (elective abortion), and her own attitude toward such a termination, might be influenced by her husband's knowledge of the pregnancy. Unprotected intercourse might be defined differently by different groups of women. Coitus interruptus, considered in some populations to be a highly effective method of contraception, might not be considered a contraceptive method by others. Other interventions, such as douching after coitus, might be reported as contraception by some women. These considerations support the need to validate questionnaires in the populations in which they are to be used.

PREGNANCY LOSS*

GENERAL CONSIDERATIONS

Pregancy loss is an adverse reproductive outcome that comprises early (or subclinical) loss (loss of a fetus before the pregnancy is recognized), clinical loss (spontaneous abortion or

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Special workgroup convened by teleconference included William Lasley, PhD; John O'Connor, PhD; and James Overstreet, MD, PhD.

miscarriage), and stillbirth. Increasingly sensitive assays continue to narrow the period during which losses go undetected; pregnancy can now be reliably diagnosed approximately 7 days postconception, or around the time of implantation. Despite these advances, the total number of pregnancy losses will be underestimated unless assays are developed that can detect preimplantation loss. Ectopic pregnancies, molar pregnancies, and voluntary pregnancy terminations (therapeutic abortions) are usually excluded from studies of pregnancy loss. Voluntary terminations can be included until the time of pregnancy termination by using survival analysis. The gestational age cutoff used to separate spontaneous abortions from stillbirths varies by country and state from 20 to 28 weeks; 20 weeks is now the most widely accepted cutoff in the United States.

Highest Level of Effort

A study at the highest level of effort should include a baseline questionnaire and daily diary, as well as obtain a biologic marker for pregnancy. This questionnaire should attempt to elicit a complete obstetrical history, by probing for early pregnancy losses, voluntary terminations (and medical reasons for termination), and molar and ectopic pregnancies, in addition to the items discussed previously in connection with menstrual dysfunction. Assessing the woman's attitudes toward pregnancy might also be useful.

Prospective followup should include a daily diary similar to that described in the section on menstrual dysfunction. The diary designed to detect pregnancy loss should also include a daily record of intercourse and use of contraception. A record of symptoms of pregnancy, including nausea, can also be useful.

Since sensitive and inexpensive assays to detect early pregnancy became available in the late 1980s, several studies have employed urinary assays for human chorionic gonadotropin

(hCG) to detect subclinical loss. Although the reduced cost of the newer assays has made them somewhat more accessible to epidemiologists, assay-based studies are still in a developmental stage, logistically difficult, and quite expensive.

The use of hCG as a biomarker for pregnancy provides information on pregnancy loss that is not otherwise detectable. Urine collection studies have identified subclinical losses in 20% to 35% of cycles in which an elevated level of hCG indicates that conception has occurred (*20,21*). Recent studies have shown that rates of conception and loss depend upon a number of design factors, including the (1) type of population recruited, (2) duration and intensity of followup, (3) specimen collection protocol, and (4) sensitivity and specificity of the hCG assay employed.

Five recent epidemiologic studies assayed urine for hCG to identify early fetal loss. These are referred to in this section by their sponsoring institutions, as follows:

- The National Institute of Environmental Health Sciences (NIEHS) (*20*).
- Johns Hopkins University (*22*).
- University of California at Davis (UC Davis) (*21*).
- California Department of Health Services (CDHS) (*23*).
- Mt. Sinai School of Medicine (*11*).

In four of the studies, all study participants collected urine daily; in the Mt. Sinai study, only a 10% subset of women collected urine during the entire menstrual cycle. Results on subclinical pregnancy are not yet available from the CDHS and Mt. Sinai studies.

The five studies attempted to recruit women who were likely to become pregnant during the study period. Therefore, women who had been sterilized, had had hysterectomies, or were using an intrauterine device (IUD) or oral contraceptive (OC) were not eligible, with one exception. The Mt. Sinai study included women who planned to stop using OCs within 6 months of entry in the study. Other entry and exclusion criteria differed between studies (Table 2-3). For example, the NIEHS study, which had the most stringent entry requirements, recruited women birth control users just before they stopped use of contraception in order to become pregnant. These women were recruited by means of newspaper advertisements, posters, and brochures. The women were quite a bit younger than those in the other four studies, and none had a history of fertility problems (Table 2-3). The NIEHS study found the highest pregnancy rate. Of 221 enrolled women, 70% achieved a clinically recognizable pregnancy.

Three of the studies (Johns Hopkins, UC Davis, and Mt. Sinai) used occupational cohorts. As with the NIEHS study, the Johns Hopkins study included women who were planning to become pregnant. The other two occupational studies included a broader cross section of women and found a lower pregnancy rate. The most representative cohort might have been that enrolled for the CDHS study, in which women were recruited from the membership files of a large health maintenance organization (HMO). In such studies, there is a compromise between statistical power and generalizability of results. Studies restricted to women with high likelihood of pregnancy yield higher pregnancy rates, but provide results for a more highly selected population. However, all biomarker studies of early pregnancy loss evaluate study populations that are somewhat different from the population at large, because women who agree to participate in such studies must be willing and able to follow

Table 2-3.—Investigations of early fetal loss, comparison of study populations.

Study Population Characteristics	NIEHS	Johns Hopkins	UC Davis	CDHS	Mt. Sinai
Participant selection	Ads*	Workers	Workers	HMO	Workers
Payment	$10/wk.	$100 total	$35/mo.	$50 total	$10/mo.†
Demographics:					
English-speaking only	Yes	Yes	No	Yes	Yes
Age range (years)	≥18	20-40	18-44	18-39	≤40
Percent >35 years of age	5	18	41	26	NA
Percent with infertility history	0	23	28	18	NA
Exclusion criteria:					
Barrier contraception	Yes	No	No	No	No§
Oral contraceptive or intrauterine	Yes	Yes	Yes	Yes	Some¶
device	Yes	Yes	Yes	Yes	Yes
Sterilized or hysterectomized	No	No	No	Yes	No
Unmarried	Yes	No	Yes	No	Yes
Currently not sexually active	Yes	No	No	Yes	Yes
Infertility or fertility problems	No	No	Yes	Yes	No
No recent menses	No	No	No	Yes	No
Postpartum or lactating	Yes	No	No	Some	No
Chronic illness					

NIEHS—National Institute of Environmental Health Sciences; UC Davis—University of California at Davis; CDHS—California Department of Health Services; Mt. Sinai—Mt. Sinai Medical Center.

*Volunteers enrolled when stopping contraception in order to become pregnant. †Ten percent subsample paid $40 per month. §Excluded only if method used consistently. ¶Enrolled oral contraceptive users only if they planned to discontinue use in order to become pregnant.

a rigorous protocol of daily urine collection and completion of daily diaries.

Two laboratories using three assays measured hCG in the five studies (Table 2-4). The UC Davis and CDHS studies both employed the Endocrine Laboratory at UC Davis, which uses an enzyme screening assay followed by a radioimmune assay (RIA) to detect intact hCG (*2,3*). By prescreening with an assay with high sensitivity and low specificity, the number of RIAs required is minimized, lowering costs and use of radioactive material. The other three studies employed the Endocrine Laboratory at the Center for Clinical Research at Columbia University. An immunoradiometric assay (sandwich assay) was developed by the laboratory (*24*) and was employed in the NIEHS study (*20*). This assay required a larger volume of urine than current assays. It used a high-affinity, polyclonal rabbit antibody to the betacarboxy terminal region of hCG; the supply of this antibody is now exhausted. Recent studies have used a monoclonal antibody, for which the supply is inexhaustible.

There are currently two RIAs employed, both of which provide sensitive and specific measurements of hCG and require less than 5 milliliters (mL) of urine for multiple replicates. The "intact" assay, used at UC Davis, uses the capture antibody B109, which has a high affinity for the intact hCG molecule, and a detection antibody (B108), which is specific for the beta subunit of hCG. This combination has high specificity for the hCG molecule, with no cross-reactivity with human luteinizing hormone (hLH). Currently, Columbia University uses a combination (combo) assay (B109/B204 and B108), which is more sensitive than the "intact" assay. In an analysis of 207 cycles that were positive for at least one of three assays tested, the intact assay was positive in 65.2% while the combo assay was positive in 88.9%. However, the combo assay is more expensive, more difficult to use, and might have lower specificity. Because some hCG is probably produced by the

Table 2-4.—Investigations of early fetal loss, specimen collection, and assay methods.

Specimens and Assays	NIEHS	Johns Hopkins	UC Davis	CDHS	Mt. Sinai
Collection methods:					
Mean number of cycles	3.2	7.8	5.0	5.5	6.8
Daily collection	Yes	Yes	Yes	Yes	No*
Urine volume (mL)	20-30†	5	5	5	30†
EIMA screen	No	No	Yes	Yes	No
Assay used:					
Intact polyclonal	Yes	No	No	No	No
Intact monoclonal	No	No	Yes	Yes	Yes
Combination (combo)	No	Yes	No	No	Yes
Adjusted to urine creatinine	No	No	Yes	Yes	No
Included tubal ligation controls	Yes	Yes	No§	Yes	Yes

NIEHS—National Institute of Environmental Health Sciences; UC Davis—University of California at Davis; CDHS—California Department of Health Services; Mt. Sinai—Mt. Sinai Medical Center. mL—milliliters.
*Ten percent subsample collected daily. †Less than 4 mL required for each replicate specimen. §Not intentionally included.

pituitary gland, no hCG assay can rule out all pituitary events. However, the combo assay might detect more of this pituitary contribution than the intact assay. It should be feasible in most instances to separate the pituitary from the trophoblastic contributions to hCG because the pituitary elevation usually occurs around the time of ovulation.

The UC Davis laboratory adjusted results for urinary concentration (using the urine creatinine level), but the Columbia University laboratory did not do so. Although such adjustment might be advisable for steroid assays, its value for hCG assays is less clear. In either case, the identification of a subclinical pregnancy loss probably does not depend on creatinine adjustment because hCG rises logarithmically after conception. However, adjustment might affect the cycle day on which the rise in hCG is determined.

The definition of subclinical loss (or "chemical pregnancy") varied between studies (Table 2-5). Four parameters must be specified in defining these events: (1) the window (or cycle days) in which the assay is run, (2) the critical value for hCG, (3) the number of days during which hCG must exceed the critical value, and (4) whether or not the days of elevated hCG are required to be consecutive. How days with missing urine specimens are handled (for example, by linear interpolation) must also be specified.

Each study found a proportion of cycles that were possibly, but not definitely, conceptive. These borderline events were usually those for which the hCG elevation was very transient or barely exceeded the critical value for a positive event. Because existing methods can detect only a fraction of all postconceptual losses, a definition of pregnancy that is less sensitive but more specific might be acceptable. The selection of the hCG cutoff for defining pregnancy, which requires balancing sensitivity and specificity, should be made with the knowledge that the

Table 2-5.—Investigations of early fetal loss, detection of occult and clinical pregnancies.

Detection Characteristics	NIEHS	Johns Hopkins	UC Davis	CDHS	Mt. Sinai
Number of participants	221	148	408	404	575
Number of cycles	707	1,157	2,040	1,858	NA
Definition of definite occult pregnancy:					
Assay window (days)	15	12	16	16	*
hCG cutoff value (ng/mg)	0.025	0.15	0.25†	0.15†	*
Days hCG greater than cutoff value	3	2	2 of 3	2 of 3	*
Required consecutive days	Yes	Yes	Yes	Yes	*
Pregnancy results:					
Clinical pregnancies	155	60	35	59	140
Clinical losses	18§	14	6	5	NA
Definite occult pregnancies	44	32	22	NA	NA
Total pregnancy rate (occult and clinical) per cycle (percent)	28	8	3	NA	NA
Loss rate per total pregnancies (percent)	31	50	46	NA	NA

NIEHS—National Institute of Environmental Health Sciences; UC Davis—University of California at Davis; CDHS—California Department of Health Services; Mt. Sinai—Mt. Sinai Medical Center. NA-Not available. ng/mg—Nanograms per milligram. hCG—Human chorionic gonadotropin.

*Only 2 days collected except for 10% subsample. †Adjusted to urine creatinine. §Includes one molar pregnancy.

pregnancy loss rate for a study can easily be inflated, even by a low false-positive rate. This is a consequence of the low rate of true positives and the huge number of cycles being assayed (*25*).

Medium Level of Effort

When daily urine collection is not warranted, urine collection can be limited to the first 2 days of menstruation of each cycle. There are data suggesting that 85% of all subclinical pregnancy losses will be identified in this way (*20*). Alternatively, inexpensive home pregnancy tests can be distributed to study participants with instructions to test urine one week after the first missed period (*26*), until bleeding resumes or pregnancy is confirmed. Although not yet widely tested in field studies, this method could prove more practical than daily urine collection. The proportion of pregnancies identified with this design is not known, but could be estimated using existing data. Both of these methods also use the baseline questionnaire and daily diary.

Lowest Level of Effort

Until recently, most studies of pregnancy loss did not attempt to estimate occult loss, but instead determined failures of recognized pregnancies. Several study designs have been employed, including (1) retrospective interviews of occupational cohorts or concerned communities (*27,28*), (2) case-control studies comparing pregnancies identified through pathology laboratories to liveborn controls (*29*) or to outpatient clinic records (*30*), and (3) prospective studies using registries of miscarriages and live births. Of these, the prospective studies probably provide the most accurate estimates of pregnancy loss if recruitment is uniform and early in pregnancy. In the United States, uniform and early enrollment might be feasible only through HMO populations in which pregnant women can be identified at the time of a positive pregnancy test or when scheduling the first prenatal appointment. Otherwise, lack of

uniformity of gestational age at which pregnancy is confirmed is likely to be a severe problem, particularly for communities with limited access to prenatal care. Early pregnancy diagnosis for high-risk pregnancies or exposed, concerned populations might also introduce bias because rates of pregnancy loss decline rapidly with gestational age.

Retrospective cohort studies require the least effort and can be less precise due to retrospective reporting of both outcome and exposure. Medical confirmation of reported loss can minimize the false-positive rate. However, the recall of early losses might be affected by the participant's knowledge of her exposure and false negatives (failure to report early losses) are not easily detectable.

A thorough baseline questionnaire should be used for any of these types of studies. Regardless of the study design, an attempt should be made to confirm all pregnancy losses, either through medical records (requiring participant consent) or by using a standardized physician interview. Pregnancy losses that cannot be confirmed are to be characterized as questionable and analyzed separately from confirmed losses.

PREGNANCY COMPLICATIONS

Pregnancy complications can be considered either as adverse reproductive outcomes to be assessed, or as risk factors to adjust or control for when evaluating other reproductive outcomes. Because of this dual status and because biologic specimens are not usually obtained for pregnancy complications, the level-of-effort classification used elsewhere in this chapter is not appropriate. Table 2-6 delineates some pregnancy complications and the risk factors associated with each end point. Identification of these pregnancy complications in a study population might be based on questionnaire responses, review of medical records, or

Table 2-6.—Examples of pregnancy complications and associated risk factors.

Complication	Frequency*	Risk Factors
Pregnancy-induced hypertension	5%	Extremes of age Low SES Nulligravidity Chronic hypertension Diabetes mellitus Kidney disease
Gestational diabetes	5%	Advanced age Obesity Family history
Hyperemesis gravidarum	1%	Young age Nulligravidity
Preterm labor	7%	Multiple gestation Urinary tract infection Uterine anomalies Extremes of age
Abruptio Placenta	1%	Hypertension
Molar pregnancy	<1%	Unknown
Ectopic pregnancy	1%-10%	Previous pelvic infection Previous ectopic pregnancy

SES-Socioeconomic status.
DES-Diethylstilbestrol.
*Approximate incidence rate.

direct evaluation of pregnant women. These complications are sometimes defined inconsistently.

Prospective evaluation of ongoing pregnancies by health providers using uniform instruments and techniques can be

performed to evaluate weight, blood pressure, proteinuria, fundal height, and plasma or serum glucose after a standard glucose load. Following pregnancies forward in time also permits the most accurate ascertainment of diagnoses of hyperemesis gravidarum, preterm labor, and placental abruption. Because of the low incidence of pregnancy complications, this level of effort would not be expected to show a difference between exposed and control populations unless the increase in adverse outcome was large or there was a large sample size.

Medical record review can be used to assess the same end points, although less reliably. Many hospitals require that a copy of the outpatient prenatal record be placed on the inpatient chart, but the level of cooperation and the completeness of such records is inconsistent. Medical records can be supplemented with a questionnaire asking women to recall pregnancy complication diagnoses. Recall errors and the possibility that women were not made aware of their diagnoses undermine the utility of using questionnaire data without medical record confirmation. Although less efficient, questionnaire data, validated with medical records when possible, can also be used in a cross-sectional design.

LACTATION DISORDERS

GENERAL CONSIDERATIONS

Because most agents present in maternal serum can gain access to breast milk, toxicant exposure of the nursing infant can be a concern. Weak bases and fat soluble compounds can be concentrated in milk; weak acids and compounds that are extensively bound to maternal plasma proteins can be relatively excluded from milk. Lactation is the main route of excretion for toxicants that bioaccumulate in maternal adipose tissue; these agents then can bioaccumulate in the infant over the duration of breastfeeding.

The potential effect of xenobiotics on milk production should also be considered. Toxicants can interfere with milk production or alter the composition of milk. Estrogenic compounds and ergot alkaloids have been used clinically to inhibit lactation, and exposure to xenobiotics with similar effects (for example, estrogenic pesticides) can also impair milk production. Some hormonal contraceptives have been shown to alter the protein-fat ratio and other components in milk. Although the clinical significance of this alteration has not been established, it is possible that a mother-child pair with marginal nutritional status might be adversely affected by such subtle changes in milk composition.

HIGHEST LEVEL OF EFFORT

Information can be collected on lactation parameters, on infant parameters, and on milk parameters. Diary information includes the timing of menses resumption.

Information should also be collected on use of tobacco, ethanol, caffeine, drugs, and medications. Diet, fluid intake, and rate of maternal weight change after delivery should be assessed. Although it is not clear that these factors will be associated with outcome parameters, data analysis should consider them as potential confounders.

Lactation Parameters

A diary can be used to give investigators prospective information on the number of feeding sessions in a day and the duration of feedings. Diaries can also be used to document when and how often supplements are given. The following parameters can be considered.

Decision to Nurse.—In the United States, 55% to 60% of women decide to nurse (*31*). A number of factors influence this

decision, including socioeconomic status, culture, and employment status. Concerns about exposure to toxicants might cause some women to decide against nursing. Finally, the condition of the infant can influence this decision; women who decide not to nurse should be asked the reason for this decision.

Pattern of Nursing.—Although breast milk is a complete food for at least the first 6 months of life, the duration and pattern of nursing varies. Distinctions are made among the following four different patterns of nursing.

Exclusive Nursing.—Only breast milk is given to the infant.

Complete Nursing.—Breast milk is the sole nutrition source. However, water, vitamins, and tastes of other foods might be given. Occasional bottle use is included in this category.

Partial or Token Nursing.—Supplementation with formula or other substitutes is used; nursing is not the primary source of nutrition.

Decisions about the extent of nursing can be made by women for different reasons, some of which are similar to the decision to nurse at all. The use of formula supplementation might be secondary to a perceived deficit in breast milk volume or to perceived infant difficulty nursing (due, perhaps to altered milk taste or impaired milk letdown). Questionnaire information about the extent of nursing should, therefore, include the reason for the chosen feeding pattern.

Duration of Nursing.—There is substantial variation in the duration of nursing. This can be influenced by hospital practices, health care provider attitudes, socioeconomic status, occupational status, and culture. The apparent satisfaction of the infant with the nursing session can affect the duration and pattern of nursing.

Additional diary information includes the timing of menses resumption.

Infant Parameters

Infant weight, length, and head circumference can be obtained periodically and plotted on normal growth curves. Deficits in these parameters in breastfed infants suggest possible adverse effects on milk volume or composition.

Milk Parameters

Xenobiotics in Milk.—The assessment of concentration of a xenobiotic in milk can be complicated by the changes in the composition of milk that occur throughout the day and throughout the individual feeding. For example, the early part of the feed, called foremilk, has a lower lipid content than the cream-like hindmilk. Measurement of xenobiotics in milk should, then, include specimens obtained at different parts of the feeding. A concentration-time curve for the agent in milk can be constructed and the area under the curve can be used as an estimate of total exposure. Estimation of the infant dose can be made; however, it should be recognized that absorption in the gastrointestinal tract is an important determinant of internal dose for the infant. Milk measurements of environmental contaminants might be difficult to interpret because of ubiquitous low-level contamination of the population and the lack of reference values.

Milk Volume and Constituents.—Milk volume cannot be measured accurately by pumping but an estimate can be made by weighing infants before and after a feeding. The amount of lipid and protein in an aliquot of milk is relatively easy to determine. It should be remembered that milk composition changes with the time of day, time during the feed, time since the last feed, gestational age at delivery, and age of the infant. These factors need to be carefully controlled for.

Medium Level of Effort

Prospective collection of lactation information using diaries, without measurement of milk parameters, will give a useful evaluation of lactation in most settings. Infant parameters can be collected in a cross-sectional manner or medical records can be reviewed to obtain this information. Pediatric records for infants can be expected to include weight and length in nearly all instances.

Lowest Level of Effort

Retrospective questionnaires on lactation can also be used, although the accuracy of information derived from questionnaires is expected to be inferior to prospectively acquired data. Twenty-four-hour recall of lactation experience might be the most accurate questionnaire-derived measure.

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Edited by

Gina J. Terracciano
Grace K. Lemasters
Robert W. Amler

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