7 Assessing the Reproductive Health of Men with Occupational Exposures*

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ABSTRACT

Male reproductive health is a product of complex synchronies among testicular, accessory sex gland, neuroendocrine, and erectile function. Hypotheses about endogenous or exogenous factors thought to disrupt one or more of these functions are amenable to human studies. The purpose of this chapter is to describe the methods used to design, conduct, and interpret such studies. Common study designs and laboratory analyses applied to study men's

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reproductive health are presented, together with special research considerations and future assessment methods.

KEYWORDS

Accessory sex glands, accessory sex gland assessment, hormone assessment, male reproductive effects, man, men, occupational exposures, productive epidemiology, reproductive neuroendocrinology, reproductive systematic review, semen analysis, sexual function assessment, sperm, study design, testes, testicular function assessment

7.1 BACKGROUND

The earliest report linking environmental exposure to adverse human male reproductive effects dates back to 1775 when an English physician, Percival Pott, reported a high incidence of scrotal cancer in chimney sweeps. This observation led to safety regulations in the form of bathing requirements for these workers [1]. Brenneke, Hertwig, Muller, and Snell were among the first to formally study effects of exposures on offspring in mice, demonstrating that irradiated males sired smaller litters and linking chromosomal abnormalities in fertilized eggs to sperm irradiation [2]. Similarly, Auerbach and Robson, and Bock and Jackson later used mice to show that chemically exposed males had reduced fertility with induction of chromosomal abnormalities and other male germline mutations [2–5]. That male-mediated reproductive harm may occur in humans as a result of toxicant exposures became firmly established only relatively recently when Lancranjan et al. studied lead-exposed workers in Romania in 1975 [6], and later in 1977, when Whorton et al. examined the effects of dibromochloropropane (DBCP) on male workers in California [7]. Since these discoveries, additional human reproductive toxicants have been identified through the convergence of laboratory and observational findings. It has also been increasingly recognized that men's nonchemical exposures, both exogenous (e.g., physical exposures such as genital hyperthermia, pressure, and radiation therapy) and endogenous (e.g., constitutional factors such as age and genetic variation), may affect men's reproductive health and capacity [8–14]. The purpose of this chapter is to provide an overview of methods used to study the effects of exposures on male reproduction and their reproductive health, with a primary emphasis on the implementation and interpretation of human studies.

7.2 INITIATION OF STUDIES

Most research on human male reproductive health has been stimulated by studies of the effects of exposures on nonhuman animals and their offspring, that is, animal models. Many research gaps remain, as the pool of potential human exposures with undetermined effects on male reproduction is vast. Consider chemical exposures. More than 99 million unique organic and inorganic substances are currently registered in the Chemical Abstract Service database of the American Chemical Society, with approximately 15,000 new substances added per day at this writing [15].

Roughly 84,000 chemicals are in commerce in the United States [16] and more than 100,000 in the European Union [17], but male reproductive toxicity has only been thoroughly investigated in a small fraction of them. Under the 2007 European regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), manufacturers and importers are required to identify and share chemical risks that are then added to a European Union registry for subsequent evaluation and public dissemination [18]. European governments have undertaken an ambitious effort to identify reproductive toxicants among a subset of chemicals produced in high volumes [19]. The bulk of this work is currently conducted by testing whole animals, although alternatives are increasingly being developed as cost and animal welfare issues attend mass testing [19–21]. Alternatives to animal testing include in vitro testing (e.g., the mouse embryonic stem cell test for early developmental toxicity or mEST), in silico (i.e., computerized) methods, such as quantitative structure–activity relationship (i.e., QSAR) models, and grouping of related substances. Statistical models, termed QSAR models, are applied to compare and contrast structurally similar chemicals by examining statistical correlations between them on qualitative variables that may affect biological activity, such as polarity, lipophilicity, and molecule size [22]. Results of animal, in vitro, and in silico tests may sometimes be used to group chemicals; the health effects (and appropriate control) for one chemical may sometimes be extrapolated to similar chemicals within the same group. Overall, these nonhuman methods are vital for early identification of potential human reproductive health hazards, but extrapolating results to humans is inherently uncertain; thus, multiple, high-quality human studies are often needed to address these uncertainties and enhance human risk prediction.

Surveillance and anecdotal observations also have led to investigations of male reproductive exposures. Studies of DBCP were initiated after informal discussions of infertility problems among wives attending a softball game [23]. The petroleum refinery industry exemplifies a profession in which the workers themselves had concerns regarding their reproductive health [24]. Work-related accidents such as contamination of a truck driver and rescue workers responding to a truck accident–related bromine spill [25] or the nuclear radiation disaster in Chernobyl [26] also have led to studies. Adverse health effects observed in case studies of high-dose accidental exposures may provide clues to potential health effects that should be studied at lower exposures. Corporations may also initiate occupational research to validate anecdotal claims as with studies on dinitrotoluene and toluenediamine [27].

Relying on anecdotes and surveillance to identify possible male reproductive toxicants, however, is haphazard. In contrast to more overt health hazards, male occupational reproductive hazards can be “silent”; this presents an obstacle to identifying emerging hazards using human populations. To illustrate, suppose hypothetically that an effect such as reversible sterility is, in fact, induced by an unsuspected male reproductive toxicant. Although this is an extremely severe effect, only the subset of nonvasectomized male men trying to achieve pregnancy (or at least having regular sexual intercourse) with reproductive-aged, noncontracepting partners during the exposure period, who underwent a diagnostic workup during the exposure period and then were informed they were sterile, would even be aware a problem exists. As another example, a broader group of workers may be privately aware of an overt

outcome such as diminished sexual function but (as with infertility) misattribute it to normal aging, and so on, and have a similar reluctance to disclose it, and thus, even after a reproductive health problem is acknowledged, it may only be known to a man's partner and, perhaps, his private physician. It is probably safe to assert, therefore, that a cluster of male reproductive health problems is far less apt to "sound the alarm" than a cluster of more commonly diagnosed and socially discussed health problems. This underscores the importance of nonhuman hazard screening efforts. Therefore, the toxicologist, the physician, the epidemiologist, the worker himself, the labor union, and the corporation will continue to be "on the lookout" for potential exposures and study populations.

7.3 STUDIES OF HUMAN POPULATIONS—DESIGNS AND CONSIDERATIONS

Animal and human experimentation on the male reproductive system have constraints, both ethical and pragmatic. As methods to reduce animal testing are evolving, so are nonexperimental, observational methods to study factors that may affect men's reproductive health or their offspring. Unlike animal studies, human studies cannot rely on random assignment of subjects to treatment or control groups or controlling all extraneous variables like diet and environment. Consequently, alternative designs and analysis methods for observational studies have been developed with the goal of controlling or minimizing biases introduced by sub- or nonrandomization. Population-based studies, broadly, are one such category of designs. The goal of sample selection in a population-based study is to represent the target human population of interest (e.g., nation, region, demographic group). To the extent sample representativeness is achieved, results of population-based studies may be considered externally valid (i.e., generalizable) for testing associations.

Epidemiological studies of occupational exposures and adverse male reproductive effects may follow several different study epidemiological (e.g., cohort, case–control, cross-sectional) and other (e.g., clinical case studies, etc.) designs. Table 7.1 describes various population-based study designs in terms of subject selection, an important determinant of how population based a study is, together with examples and the potential advantages and disadvantages of each design. These will be discussed at length later in the chapter.

In any study of occupational exposure and adverse reproductive effects, however, there are several challenges that must be considered. Designing reproductive studies of male populations that are externally valid presents some unique challenges. Selection bias may occur when enrollment or study completion varies between men, with versus without known or suspected reproductive health problems. Observational male reproductive studies may involve collection of questionnaires, blood or urine for neuroendocrine and other measurements, semen analysis, and sexual function analysis, either alone or in combination. Questionnaires are the least expensive and least invasive, and so may be more readily accepted. Male reproductive questionnaires, however, typically contain sensitive items (i.e., sexual function and habits, lifestyle, and disease histories may be requested) that may limit their acceptability for some people. Men with reproductive health concerns may be disproportionately

TABLE 7.1
Selected Design Elements for Various Human Male Reproductive Study Designs: Examples, Advantages, and Disadvantages

Study Design	Description	Example	Advantages	Disadvantages
Prospective cohort	*Subject selection*: based on defined group membership (e.g., common industry, birth year, region, etc.) *Aim*: follow entire group forward in time to track emergent exposures and outcomes (e.g., disease). Outcomes are later compared among prior exposure and nonexposure subgroups and with population-based regional or national statistics.	The Japanese atomic bomb survivor cohort has been followed over time for the development of cancers, including cancers of male genitalia, in order to calculate the relative risk (RR) of these cancers associated with radiation dose.	- Typically includes most members of the population of interest (no sampling), so extremely population based. - Permits calculation of incidence and RR.	- Time and $ costs relatively high unless outcomes manifest quickly (e.g., semen alterations). - Inefficient for rare diseases. - Attrition issues. - Special ethical issues pertaining to prospective follow-up.
Retrospective cohort (AKA historical cohort, nonconcurrent prospective)	*Subject selection*: based on defined group membership at some designated earlier point in time. *Aim*: follow entire group over historical time (retrospectively reconstructed) to track emergent exposures and outcomes. Outcomes are then compared among prior exposure and nonexposure subgroups and with concurrent population-based regional or national statistics.	Past paternal occupational group(s) recorded on birth certificates might be linked to registries to calculate the RRs of birth anomalies among offspring associated with occupation type.	- Time and $ costs relatively low. - Permits calculation of incidence and RR if group is population based. - Efficient for outcomes posited to manifest long after exposure (e.g., birth defects, cancer).	- Historical records may not contain variables of interest or may record them in insufficient detail. - Questionnaire responses of cases and controls regarding historical exposures subject to differential recall bias. - Does not permit calculation of incidence unless group is population based.

(*Continued*)

TABLE 7.1 (CONTINUED)
Selected Design Elements for Various Human Male Reproductive Study Designs: Examples, Advantages, and Disadvantages

Study Design	Description	Example	Advantages	Disadvantages
Case–control (AKA case–referent)	*Subject selection*: based on the outcome's (e.g., disease's) presence (case) or absence (control). When case and control groups are drawn from a cohort study, it is a "nested case control" design (similar to retrospective cohort). *Aim*: using historical exposure and history information, compare prior exposure vs. nonexposure history of cases and controls.	Past workplace exposures of infertile (cases) and fertile (controls) male workers are compared and infertility risk is estimated by calculating the odds ratio associated with exposure.	- Time and $ costs relatively low. - Efficient: requires smaller sample sizes; often used for rare diseases. - Nested cases and controls reduce potential bias.	- Does not permit calculation of incidence. - Generates odds ratios that approximate RRs only for rare outcomes. - Considerable bias potential attributed to use of inappropriate control group (bias addressed by use of nested cases and controls).
Cross-sectional (AKA prevalence)	*Subject selection*: based on defined group membership (e.g., common industry, birth year, region, etc.). *Aim*: using "snapshot" of current outcome and exposures, compare exposure vs. nonexposure histories of those who currently do vs. do not have the outcome.	A group of workers' short-term exposures are assessed using urine samples and current sexual function scores by questionnaire. Prevalence of low scores and association between scores and exposures are described.	- Time and $ costs relatively low. - Permits calculation of prevalence if population based. - Suited to collecting detailed data (surveys, exams, and biomarkers) for outcomes not routinely monitored. - Can be repeated in the future to develop a prospective cohort.	- Noninformative regarding whether exposure preceded disease (or vice versa) unless exposure is acute and outcome is immediate. - Does not permit calculation of incidence. - Hypothesis generating, exploratory design.

(*Continued*)

TABLE 7.1 (CONTINUED)
Selected Design Elements for Various Human Male Reproductive Study Designs: Examples, Advantages, and Disadvantages

Study Design	Description	Example	Advantages	Disadvantages
Clinical trial (AKA, clinical experiment)	*Subject selection*: based on defined group membership or a convenience sample (e.g., patients). *Aim*: randomly select participants into intervention or nonintervention groups with prospective follow-up to compare outcomes between groups who did and did not receive the intervention.	A group of men are randomized as to whether or not they will consume "Medication X" vs. a placebo to test the effect of the treatment on erectile function.	- Ideal to assess possible cause–effect relationships. - Randomization minimizes confounding bias.	- Ethical and feasibility considerations limit scope of exposure interventions acceptable for human experiments. - Often conducted on small, potentially underpowered sample sizes.

motivated to participate and so less deterred from providing sensitive questionnaire information and samples (e.g., semen, blood) or complying with study requirements (e.g., abstinence before sample collection, attending multiple study appointments). High participation rates are desirable to improve the representativeness of study samples, especially when participants and nonparticipants differ systematically on the reproductive health or exposure factors under study. Such high participation rates are not generally achieved in studies of men's reproductive health. Recruitment is often more successful when populations of interest are defined more narrowly (e.g., clinics, industries), as the pool of participants may be more uniformly motivated and recruitment efforts more targeted. For instance, participation rates for community-based semen quality studies are generally very low [28], but low refusal rates have been achieved among motivated men from narrower source populations, for example, military settings [29] and men whose partners previously had spontaneous abortions [30]. This illustrates a commonly encountered design dilemma when achieving a high participation rate requires a generalizability trade-off in terms of using a more motivated and narrowly defined source population. Even within narrowly defined target populations, individual constituent clinics and companies targeted for recruitment may opt out, or their inclusion may not be feasible; hence, convenience samples are often used but not without potentially incurring more loss of generalizability. Engaging potential participants involves up-front study budget and timeline investments to clearly present the project and "market" the importance of its goals, with emphasis on the vital role of high participation to producing valid results; reasonable financial compensation for time, travel, child care, work hours lost, and so on; and maximizing the convenience and privacy of participation. Mobile vans and shipped semen samples (discussed further in Section 7.10), for example, improve the convenience and privacy of delivering semen samples.

For an observational study to be externally valid, it must also be internally valid or free from internal biases and nonrandom error. Information biases, such as recall, reporting, and misclassification bias, can influence the internal validity of reported and record-based information and cloud interpretation of laboratory-based sample measurements. Recall bias is a memory-related bias that occurs when those who have experienced events (e.g., illnesses) have altered recall (typically keener or even exaggerated exposure recall) when queried in retrospect compared with those who have not. This phenomenon is not unique to reproductive health studies and may happen whenever reported information (e.g., study questionnaires, interviews, and patient-reported medical histories) are used. For example, a person who has experienced an adverse outcome may report an exposure more readily than one with no history of reproductive dysfunction. As Levin [31] describes, a couple that has recently experienced a stillbirth or congenitally malformed child will be more inclined to search for a previous toxic exposure as the source. In an analysis of recall levels in a time-to-pregnancy (TTP) study, most men (74%) and their female partners agreed fairly well on TTP within + 2 menstrual cycles [32]. Men's recall of TTP varied with the number of pregnancies fathered, self-assessed reporting confidence, prenatal diethylstilbestrol cohort membership (positively), planning of said pregnancy, current marital status to said partner, and his education. The authors suggested that recall among men who took part in TTP studies may be heightened in comparison

with that of men from the general population. Reporting bias may occur when participants are reluctant to provide information on sensitive topics.

Although blood sampling is a widely accepted medical practice, it is an invasive procedure. In male reproductive studies, hormone levels are often obtained from blood samples, but endocrine profiles may not necessarily reflect the status of the male reproductive system [33]. Semen analysis provides information on spermatogenesis, accessory sex glands, and sperm cell motility [34]. Studies using semen samples might require the participants to have the capacity to produce samples by masturbation, raising concerns regarding participation bias should a toxicant under study concurrently affect the ability to produce ejaculate [35]. Additionally, this procedure employs complex scientific equipment and methodologies [36]. Cohort semen studies that combine all these analysis approaches provide the most complete assessment and thus the greatest likelihood of detecting adverse reproductive effects. However, such studies are expensive, complex, and necessitate a team approach minimally requiring (1) an andrologist, (2) an epidemiologist, (3) an industrial hygienist, (4) a physician, and (5) a statistician [37].

Misclassification is another threat to internal validity. Nondifferential misclassification may occur when groups being compared are equally likely to be misclassified with regard to either their exposure or disease, whereas differential misclassification occurs when the groups being compared are not equally likely to be misclassified. Nondifferential misclassification (e.g., random memory errors) typically biases results toward finding no association, that is, "the null," whereas differential misclassification can potentially bias results toward erroneous findings of either no association or a false association. For example, differential misclassification could result were respondents with sensitive reproductive health–related exposure, or disease histories, systematically skip or inaccurately report their histories on questionnaires or in interviews. Differential misclassification can also magnify the observed association when there is a true (but lesser) association.

Confounding bias can occur when both an outcome and an exposure vary according to yet another factor(s), and adequate adjustment for the confounder is not achieved by the study design and analysis. For example, maternal age could hypothetically confound an unadjusted analysis of paternal age and spontaneous abortion. This is because advanced maternal age is associated with both increased spontaneous abortion risk and older partner (paternal) age.

Use of secondary data sources (e.g., preexisting health history, personnel, surveillance, registry, or company records) originally collected closer in time to health and exposure events can mitigate potential recall bias. For studies that are resource intensive and require large sample sizes, use of secondary data may offer a less costly means to achieve adequate statistical power. One limitation of using secondary data is that information of interest may not have been recorded, and this particularly holds true for "silent" male reproductive conditions among groups outside of clinic settings. Secondary data sources are useful sources of population-based data for studies or surveillance of rare events that require large, otherwise costly, samples, such as birth defects. In the United States, however, medical birth records frequently do not record important information such as parental occupation, whereas this type of data is routinely collected in birth records of European countries. Also,

documentation of potential confounders (e.g., alcohol use, drug use, smoking, or job status) may be limited or, when available, categorized too broadly. For example, pesticide exposure of such heterogeneous professions as crop farming and a fishery husbandry may be very different although these two groups may be classified together in existing databases. Alternatively, a researcher may elect to use questionnaires or questionnaire-based interviews or, less frequently, clinical assessments or sample collections (biological or environmental sampling) as the primary source(s) of data. This strategy provides more control over what, when, and how data will be collected, advantages to be weighed against the potential for recall bias and unacceptable nonparticipation levels (and resultant biases and costs) that sometimes hamper primary data collections. Primary data may be collected exclusively or in conjunction with secondary data to augment and validate the primary or secondary data sources. The goal of conducting population-based sampling, data validation, controlling for confounders, and achieving a high participation rate is to produce valid, replicable conclusions about potential human reproductive toxicants via nonexperimental methods.

General strategies to control or reduce bias in studies are available, such as precise definition and ascertainment of larger target populations of interest, random or otherwise representative sampling schemes, achievement of adequate statistical power and a high participation rate, control of confounding, and application of advanced analysis methods to account for residual biases (e.g., sensitivity analysis or Bayesian methods).

7.4 COHORT STUDIES

Reproductive cohort studies evaluate the frequency of adverse outcomes among a group defined by common characteristics (e.g., demography, geography, exposures) by following them over time.

In such a study, baseline data are collected, and individuals are followed longitudinally, either prospectively or retrospectively, for a specific reproductive outcome. In TTP studies, for example, cohorts of couples attempting to become pregnant are followed either prospectively or retrospectively until pregnancy is achieved. Men may be informants for prospective TTP studies, particularly when paternal behaviors or exposures are thought to affect the outcome of interest. Similarly, retrospective TTP cohorts may be constructed on the basis of the male partner's exposures. Results of prospective and retrospective TTP studies may differ, as pregnancy attempts are the usual sampling unit for prospective studies, whereas the pregnancy itself is the usual sampling unit for retrospective studies [38]. In general, less recall bias is anticipated among prospective than retrospective cohorts, and for retrospective cohort studies, less recall bias among shorter-term than longer-term studies. One example of a short-term, prospective cohort study of men involved "summer hire" pesticide applicators [39]. This example illustrates the importance of selecting appropriate variables for a prospective study design. Individuals were evaluated at the beginning of the season before they started working with pesticides and at the conclusion of the spraying season 2 months later. If semen analyses are conducted to predict reproductive outcome, however, correct timing is needed. Since the time for spermatogenesis and delivery of mature sperm to the ejaculate is approximately 72 days, if primary spermatogonia

were affected by exposure, this would not be observed in a time frame that covered less than 80 to 90 days. Thus, a study of summer-hire workers could not make valid conclusions regarding the effect on spermatogenesis of a 2-month exposure among pesticide applicators. An example of a longer-term retrospective cohort study was the 1989 Vietnam experience study, in which military veterans were grouped according to whether or not they had served in Vietnam from 1967 to 1972 [40]. This study was able to detect subsequent differences between the groups in semen quality and TTP but revealed little about the reproductive health of the individuals at the time of exposure.

7.5 CASE–CONTROL STUDIES

Case–control or case–referent studies involve comparing the frequency of toxic exposure of men who have experienced reproductive dysfunction to those without such a medical history [31]. Case–control studies provide an efficient design to detect the association of rare outcomes with toxic exposures. For example, Nassar et al. [41] applied the case–control study method to examine a posited association between parental exposure to endocrine-disrupting chemicals (EDCs) and hypospadias among their offspring. Hypospadias is a birth defect that involves the urethra of the penis. Cases were obtained from a state birth defects registry in Australia and controls were a random sample of noncases from birth records from the same state. Maternal and paternal occupations, as well as information on other potential confounders and covariates, were obtained from birth records. Maternal and paternal exposure to EDCs was estimated for the various occupations. Use of this approach permitted separate estimates of the odds of hypospadias given maternal and paternal prenatal EDC exposures. Other similar examples are cited in a previous systematic review and meta-analysis on the same topic [42]. Case–control studies are, however, subject to considerable bias when cases do not arise from the same population as controls. When cases and controls are selected from existing cohorts, such designs are described as "nested" case–control studies; this approach lowers the risk of bias as both cases and controls are drawn from the same population, plus it is cost-effective.

7.6 CROSS-SECTIONAL STUDIES

A cross-sectional study provides a "snapshot" of men's exposures and reproductive outcomes as they exist at a fixed point in time. In contrast with cohort and case–control studies, a purely cross-sectional study does not include either prospective or retrospective exposure or outcome information. Such data may be particularly useful to explore relationships between acute or short-acting exposures and more transient endpoints (e.g., sperm counts). Cross-sectional studies are often less expensive to implement than other study designs and so are often used to examine hypothesized relationships. Even when high-quality, population-based cross-sectional studies may suggest associations, because of the immediate temporal nature of cross-sectional data, these studies are less informative about causality than cohort studies. Cross-sectional data can be used to estimate the prevalence, not incidence, of an outcome.

7.7 CLINIC-BASED STUDIES

Case studies typically involve the report by a physician of clinic or hospital patients exposed to potentially toxic agents. These reports involve the evaluation of individuals, groups of men with the same exposure (e.g., occupation, lifestyle), or clinical treatment after accidental exposure. While such reports rarely provide a definitive relationship between exposure and male reproductive effects, they can serve as sentinel reports that initiate further studies.

Some case studies provide unique information that would not be observed by using other study methods. One such study of a firearms instructor [43] provided possibly the best demonstration of the effect of lead on sperm. The instructor had fathered one son but became infertile as a result of work exposure that elevated his blood lead concentration to 88 μg/dl. During the next 3 years, the exposure was decreased, and the man was placed on chelation therapy. His sperm count increased as his blood levels decreased, and he later fathered another child after his blood level of lead decreased below 30 μg/dl. Similarly, after men exposed to high levels of kepone in the work environment were treated with cholestyramine to offset the toxic action of kepone, their sperm count and sperm motility increased accordingly [44].

Clinical (i.e., hospital and clinic based) studies of treatment outcomes (e.g., cancer, fertility [45]) vastly outnumber studies of men's exposures in the clinical literature on male reproduction. However, the advent of clinical data and specimen biorepositories that include sperm offers potential opportunities to expand research on potential targets and mechanisms of adverse male-mediated reproductive effects in humans. The National Institutes of Health/National Institute of Child Health and Human Development Cooperative Reproductive Medicine Network Biorepository of data (i.e., clinical, demographic, and laboratory data) and samples (i.e., serum, saliva, and sperm) promises to yield "a unique platform to assess developmental outcomes from conception to birth" [46].

7.8 SURVEILLANCE

Surveillance of human male reproductive health encompasses monitoring levels of adverse reproductive health effects in male populations and adverse effects on their offspring. Large-scale surveillance programs are ideally population based and thus describe information useful for tracking rates (e.g., incidence, prevalence) and ratios (e.g., standardized fertility, birth, and sex ratios) over time and comparing rates and ratios within and between populations. Examples of the types of surveillance systems that capture relevant outcomes for men's reproductive health monitoring and studies include sexually transmitted disease, cancer, births, and adverse birth outcome tracking. These systems are primarily registry based and maintained or supported by government agencies. Use of these systems to track male reproductive outcomes among subgroups of exposed men (e.g., occupational groups, etc.) or to study exposure–reproductive disease relationships is limited by the extent such systems fail to capture men's exposures. For instance, Fitzgerald et al. [47] found that "father's usual occupation" is listed on birth certificates by only a third of states in the United

States. Because exposure variables available from surveillance databases are often broad, careful attention to the appropriate use and interpretation of such data is indicated. Brender et al. [48] found agreement on paternal occupation between reported (maternal interview) and recorded (birth certificate) data sources 63% of the time.

Surveillance to monitor male reproductive health among targeted population subgroups (e.g., occupational, clinical) is also conducted. In the United States, a surveillance strategy for evaluating men working with known male reproductive toxicants was proposed and conducted by a team from the University of California [49]. However, this program had many problems and was eventually discontinued [50]. While this first attempt was discouraging, surveillance remains warranted as chemicals such as lead and ethylene glycol ethers remain in the US workplace, posing a potential hazard to the reproductive health of the male worker. Better surveillance is needed to monitor those working with these and other occupational toxicants. Addition of biological markers of reproduction and semen characteristics and evaluation for use of occupational exposure data from existing sources (e.g., birth certificates) are potential activities to enhance human surveillance [51]. Surveillance of reproductive health findings across multiple studies may also be conducted in the form of systematic reviews or meta-analyses, such as multinational efforts to monitor for the existence of declines in men's sperm counts [52].

7.9 SYNTHESIS

Narrative reviews, the traditional means of synthesizing information from multiple studies, continue to dominate the human male reproductive review literature. Increasingly, however, more formal and less ostensibly subjective methods to synthesize studies, such as systematic reviews, meta-analyses, and pooled analyses, are also being published. Narrative reviews provide an overview of past study findings from the perspective of the author(s) with interjection of their opinions on the relevance and quality of individual studies, as well as their interpretation of the body of evidence and its implications. A number of recent narrative reviews have, for example, focused on the relationship between men's body mass index (BMI) and various reproductive health outcomes including infertility [53–58]. Narrative reviews are often subjective in approach. Considered together, though, they represent a cross section of the perspectives of various subject matter experts and offer a platform for dissemination of emerging and novel ideas about potential mechanisms and implications.

Systematic reviews are a more formal approach to synthesizing information from multiple studies, since the content is more explicit and exhaustive. Study search strategies are documented and should be replicable and typically use relevant electronic search engines (e.g., MEDLINE, EMBASE, Biological Abstracts, PsycINFO, and CINAHL). Efforts to include all relevant studies for consideration may involve active discovery of studies in less accessible sources, such as results embedded in unpublished documents and reports. For example, MacDonald et al. [59] published a systematic review of the research literature on the relationship between BMI and men's semen parameters and reproductive hormone levels. They reported adherence to "Quality of Reporting of Meta-analysis of Randomized Controlled Trials and

Observational Studies" standards (QUOROM, MOOSE) [60,61]. The authors concluded that there was strong evidence of an inverse relationship between BMI and testosterone, free testosterone, and sex hormone binding globulin (SHBG). While arguably much less subjective than narrative reviews, the reporting characteristics of systematic reviews have been evaluated and systematic reviews conducted by different authors on the same topic can also be inconsistent [62].

Meta-analysis is a statistical approach for combined analysis of results from multiple distinct but comparable studies. The studies are typically selected for inclusion based on those identified through a systematic review. Meta-analysis is increasingly being applied to observational studies of semen quality and male-mediated birth outcomes. MacDonald et al. [59] coupled their previously described (above) systematic review of BMI, hormones, and semen with a very small (five-study) meta-analysis of semen parameters, finding no evidence of a relationship of semen with BMI. It is noteworthy that meta-analyses conducted with a small number of studies may have very low power, even when the number of subjects across studies is large and the effect size is substantial [63]. Meta-analyses may be undertaken with the primary "analytic" goal of identifying and estimating differences among study-specific effects or, more controversially, with a "synthetic" goal of estimating an average effect across studies [38]. Many texts have been written on procedural and statistical implementation of meta-analyses, and a number of common and specialized software packages are capable of performing it [63]. Most commonly, meta-analysts use results presented by other investigators as their data (i.e., means, standard errors, confidence limits), and thus, meta-analyses may be subject to the same biases as the constituent studies. Differences between methods and participants are generally inherent in human observational studies, and deciding where these study differences fall on a continuum between "fixed" and "random" is important for meta-analysis design and results interpretation. If all studies are so similar as to be "functionally identical" and the goal is to estimate a common effect size for those studies rather than to generalize, then fixed effects may be in order [63]. Alternatively, if the studies were conducted independently by different researchers and hence likely not "functionally identical," then random effects may be more appropriate. Meta-analyses with fewer studies, or where random versus fixed effects is less clear, may opt to present results of both random- and fixed-effects analyses. While large differences between studies may render them noncombinable, it may be argued that, when relatively slight, inherent study diversity may temper biases in the individual studies, that is, if the biases of constituent studies were not overwhelming and did not alter results in the same direction. It is critically important, therefore, that constituent studies be of sufficient quality for inclusion. Also, the level of similarity of constituent study populations and methods must be appropriate, given the primary goal(s) of the meta-analysis. Meta-analyses are subject to an extra layer of potential biases related to study selection. Measures to reduce these biases (or the appearance of bias) include replicable and documented systematic reviews of the literature for relevant studies, plumbing alternative sources for analyses studies, transparent documentation of rationale(s) for study exclusion, identification and screening of constituent studies for inclusion by parties not involved in those studies, appropriate weighting (fixed vs. random) according to study similarity, and adjustment for bias in the analysis. The subjective

nature of these activities is a reason that "synthetic" goals may be considered controversial [38]. Thus, meta-analysis "consumers" interested in average effects must be particularly mindful of these potential biases. Meta-regression offers a method of examining associations between variables across studies but has much larger sample size (i.e., number of studies) requirements than meta-analysis.

Pooling of the actual data across studies, as opposed to combining summary statistics as is done for a meta-analysis, is generally the most highly preferred method of data synthesis, when feasible. Continuing with the BMI research example theme, Aggerholm et al. [64] combined data from five population-based environmental studies of the relationship of BMI to male reproductive hormone levels and semen quality into one large database ($N = 2139$). The authors reflected on the degree of homogeneity of the study populations and the comparability of sample collection and laboratory analysis protocols, all key considerations to be weighed before pooling studies.

7.10 ASSESSMENTS OF MALE REPRODUCTIVE HEALTH

Toxicants can attack the male reproductive system at one of several sites, or at multiple sites. These sites and the assays associated with their respective functions are discussed individually. This does not necessarily indicate, however, that there exists an absolute one-to-one relationship between a particular measurement and the associated site of action. These sites include the neuroendocrine system, the testes, accessory sex glands, and sexual function.

The establishment of a male reproductive profile for assessing reproductive potential for both individual and population investigations is essential. The same profile can be used for both types of studies, but there are some basic differences in methodology. The assessment profile illustrated in Table 7.2 is being used by the National Institute for Occupational Safety and Health to assess populations exposed to potential reproductive toxicants. Differences between assessing the individual versus the population will be noted. A summary of assessments and specific methodologies follow. If individual data (vs. population comparisons) are to be used, care should be taken to compare the results with the normal range of results of the laboratory conducting the analysis and not published values. If a population-based study is being conducted, a concurrent comparison cohort must be used and the analyses should be

TABLE 7.2
Endocrine Profile for Assessing Reproductive Toxicant Effects

	Fluid for Measurement		
Hormone	**Saliva**	**Blood**	**Urine**
Luteinizing hormone		X	X
Follicle-stimulating hormone		X	X
Inhibin B		X	
Testosterone—Total		X	X
—Free	X	X	

TABLE 7.3
Semen Characteristics—Reference Limits (5th Centiles)

Semen Characteristics	Lower Reference Limit (95% Confidence Interval)
Semen volume (ml)	1.5 (1.47–1.7)
Total sperm number (10^6 per ejaculate)	39 (33–46)
Sperm concentration (10^6 per ml)	15 (12–16)
Motility (%)	40 (38–42)
Vitality (live spermatozoa, %)	58 (55–63)
Sperm morphology (normal forms, %)	4 (3.5–4.0)

Source: World Health Organization, *WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th edition*. World Press, Geneva, Switzerland, 2010.

blind to exposure status. Table 7.3 provides the World Health Organization (WHO) reference values for various semen parameters [65].

Table 7.4 provides examples of occupational exposures that have been shown to have negative effects on one or more sites of male reproduction. The most effective data collection is achieved by establishing a temporary laboratory near the worksite for blood collection and designed such that the semen samples can be conveniently submitted. Studies with multiple study sites or long recruitment periods may make establishing a temporary laboratory impractical. In this case, blood can be collected by a local nurse or clinic and serum can be shipped to the analytical laboratory. The semen sample can be collected, placed in a cold (not frozen) container, and shipped to the andrology laboratory [66]. When semen is shipped in this manner, sperm motility and viability measures are compromised, but the other semen parameters can be assessed [67].

7.11 NEUROENDOCRINE SYSTEM

The endocrine and nervous systems work in concert to coordinate the function of the various components of the reproductive axis, drawing upon inputs that are external (e.g., sexual cues, temperature) and internal (e.g., checks and balances between endocrine tissue function, metabolic status). The reproductive endocrine status of the male can be assessed by measuring the hormones in the blood, urine, or saliva, depending on the hormone. The principal hormones of interest for assessing the effects of reproductive toxicants in men are luteinizing hormone (LH), follicle-stimulating hormone (FSH), inhibin B, and testosterone.

Since the circulating profile of LH is pulsatile, the status of this hormone for the individual, if measured in blood, is best estimated in serial samples. The pooled results of three samples collected at 20-min intervals will provide the best estimate of mean concentration [68]. Yet, multiple blood draws often result in poor participation rates of workers. If a population is being evaluated, a single blood sample per individual may suffice [69]. Alternatively, an integral of its pulsatile secretion may be obtained by measuring LH in urine [70].

Circulating FSH levels are not as variable as those for LH. This is attributable in part to a longer circulating half-life for FSH compared to LH. Thus, analysis of a

TABLE 7.4
Examples of Workplace Exposures Affecting Reproductive Health

Site of Action	Examples
	Neuroendocrine
Hormone profile	Insecticides [71], lead [72,73]
	Testicles
Sperm concentration	Lead [72,74], diesel exhaust [75], pesticide [76], bisphenol A [77]
Sperm morphology	Insecticides [71], lead [74], carbon disulfide [78], pesticide [76], bisphenol A [77]
Sperm genetics	Phthalate [79], styrene [80], OP pesticides [81], carbyl [82], fenvalerate [83], lead [74]
	Accessory Sex Glands
Toxicant in semen	Lead [73], trichloroethylene [84], boron [85], cadmium [86]
Semen volume	Lead [73], organophosphate [87]
Sperm viability	Carbon disulfide [78], bisphenol A [77], lead [74]
Sperm motility	Insecticides [71], diesel exhaust [75], lead [74], carbon disulfide [78], phthalate [79], pesticide [76], bisphenol A [77], fenvalerate [88]
	Sexual Function
Libido	Carbon disulfide [78], bisphenol A [89]
Erectile function	Bisphenol A [89], bicycle saddles [10]
Penis sensitivity	Bicycle saddles [90]
Ejaculatory function	Bisphenol A [89]

single blood sample for an individual will provide a more reliable estimate of FSH than for LH. FSH can also be measured in urine for the sake of convenience. Neither gonadotropin is exuded into the saliva.

The variability of inhibin B levels secreted by Sertoli cells into the serum is also nominal. Therefore, inhibin B levels can be assessed with a single serum sample. Inhibin B cannot be measured meaningfully in urine or saliva.

Approximately 2% of circulating testosterone is free, with the remainder bound to SHBG, albumin, and other serum proteins. The free circulating testosterone is the active component and therefore provides a more accurate marker of physiologically available testosterone than does total circulating testosterone under conditions when SHBG concentration or binding may be variable [68]. The circhoral fluctuations of circulating testosterone levels, like those for LH, are significant. Estimates of free and total testosterone can be determined in single blood samples but are greatly improved by assaying multiple blood samples and pooling the results.

Serum levels of total and free testosterone can be measured directly. However, serum-free testosterone concentrations are more accurately determined by calculating them from serum concentrations of total testosterone, SHBG, and albumin and association constant [91]. Alternatively, a single measurement in urine of testosterone after sample hydrolysis or of testosterone metabolite (e.g., androsterone,

etiocholanolone, or testosterone glucuronide) provides a convenient index of total testosterone [92]. Quantifying testosterone in saliva affords a convenient alternative to blood sampling while providing a measure of the unbound, biologically active component of circulating testosterone [93]. If measuring steroid hormone metabolites in urine, consideration should be given to the potential that the exposure being studied may alter the metabolism of excreted metabolites. This is especially pertinent since most metabolites are formed by the liver, a target of many toxicants. Lead, for example, reduces the amount of sulfated steroids that were excreted into the urine [94]. Precision of urinary measurements is improved by normalizing urinary flow rate (concentration) by adjusting for urinary levels of creatinine or osmolality.

Circulating levels for the reproductive hormones become elevated during night as the male enters puberty. In men, secretion of testosterone and inhibin B maintains this diurnal pattern through adulthood, with peak values in early morning and declining toward late afternoon [95]. This pattern appears to be driven by sleep, not a circadian rhythm [96]. Thus, samples for assessing testosterone and inhibin B should be collected at approximately the same time of day to avoid variations owing to diurnal secretory patterns.

In summary, Table 7.2 lists the primary hormones for assessing reproductive toxicity effects in men. FSH, LH, inhibin B, and testosterone can all be evaluated in a population-based study by assessing the hormone levels in a single blood sample from each man, preferably at about the same time of day. A wide variety of potentially toxic occupational exposures, including DBCP [97], phthalate [98], stilbene [99], trichloroethylene [100], fluoride [101], bisphenol A [102], radiation [103], and sedentariness [104], have been reported to alter serum levels of one or more of these hormones. Recent publications representative of the literature describe the effects of various pesticide and lead exposures on serum levels of all four hormones [105,106]. Alternatively, urine samples typically represent a more convenient way to measure the gonadotropins and testosterone in populations. Few population studies of men have assessed occupational exposures on endocrine effects measured in urine or saliva [107,108]. For the study of an individual, three blood samples collected 20 min apart or urinary assessment will improve the estimate.

7.12 TESTES

Semen analysis provides a useful profile of the function of the male reproductive system. The WHO [65] has published reference ranges for semen parameters and these are provided in Table 7.3 as general information. The various measurements that are routinely used in the assessment of occupational exposure are presented in the list on next page. Specific instructions should be provided to each man to ensure that the semen sample is properly collected by masturbation after a set time of abstinence (usually 2–3 days) and delivered to the laboratory within 1 h from the time of ejaculation. The men should be instructed to maintain the semen at room temperature, avoiding any temperature shock to the sperm cells. At the time of collecting the semen sample, each subject should record the duration of abstinence, time of semen collection, and any information regarding sample collection loss or spillage. Providing a label on the jar facilitates the recording of this information.

Semen analyses can be conducted in two phases. The initial evaluation of the sample should be conducted when the sample arrives at the laboratory (or field site) and should consist of recording the temperature, turbidity, color, liquefaction time, and volume of the semen. Temperature shock to the semen sample can affect many sperm parameters. An inexpensive temperature logging monitor on the collection jar is useful to determine the temperatures to which the semen has been exposed since collection. Motility assessments, viability estimates, sperm counts, the preparation of slides, and preservation of seminal plasma should also be conducted at this time. Sperm motility should be assessed objectively either with computer-assisted sperm analysis (CASA) or by counting nonmotile cells in an aliquot, then counting all cells in a separate aliquot that has been heated to immobilize the sperm. Percent motile is the total in the heated aliquot minus the nonmotile sperm in the first aliquot. CASA can be conducted on-site with the fresh ejaculate or video recorded for future analyses. If CASA is used, several sperm motility variables can be measured (see list below). These variables provide useful information on the progression of sperm cells (curvilinear velocity, straight-line velocity, and linearity) as well as the sperm motility pattern. Sperm motility characteristics should be measured in a chamber at least 10 μm deep in order for the sperm to move freely in all planes. Morphologic and morphometric analyses of sperm on slides may be conducted at a later time.

Semen Profile for Assessing Reproductive Toxicant Effects

- Sperm concentration
- Sperm viability
  - Vital stain
  - Hypo-osmotic swelling
- Sperm motility
  - Percent motile
  - Curvilinear velocity
  - Straight-line velocity
  - Linearity
  - Lateral-head amplitude
- Sperm size and shape
  - Morphology
  - Morphometry
- Sperm genetics
  - DNA stability
- Semen parameters
  - pH
  - Volume
  - Marker chemicals from accessory glands
  - Toxicant or metabolite concentration

Measurements of sperm motility and velocity should be conducted using a microscope stage warmed to 37°C. An attempt to record 200 motile sperm per sample is desirable if one is interested in the distribution of velocity measurements, but 100 motile sperm will suffice if means are to be compared. When assessing motility, one

should avoid "hunting" for motile sperm. All fields examined or searched should be included in the calculations; therefore, assessing a certain number of arbitrary fields is advised. Whole semen should be used for measuring sperm motility. If a CASA system is being used for velocity estimates, the number of sperm per field should be reduced to minimize cell collisions. Using a 10- to 20-μm-deep chamber, the sperm concentration should be less than 40 million/ml. Diluents (including seminal plasma), however, alter sperm velocity up to a dilution of approximately 1:1. The current recommendation for CASA of sperm velocity is to dilute all samples to one part semen in one part iso-osmotic buffer. If this dilution does not reduce the sperm concentration below 40 million/ml, then an additional dilution in the same buffer should be performed on those concentrated samples [36]. Thus, two recordings should be made: whole semen for percent motility and diluted sperm for sperm velocity.

Sperm viability may be determined by vital stain [109] or by hypo-osmotic swelling (HOS) assay [110]. The HOS assay determines the structural and functional integrity of the cell membrane [111].

Sperm morphology should be estimated on fixed, stained semen smears. During the past 30 years, several schemes have been presented for the assessment of normal and abnormal sperm morphologies. Variations in sperm size and shape are not distinct, but rather a continuum. This provides a challenge within and especially among laboratories to establish a repeatable system for morphologic classification [112–115]. Since 1980, the WHO has adopted different sperm morphology classifications several times. Currently, there are two widely accepted classification systems, WHO 3rd Edition [116] (often called traditional morphology) and WHO 5th Edition (often called strict morphology) [65]. The main difference between these classification systems is how they classify a "borderline normal" sperm; normal with traditional scheme, abnormal with strict scheme. With recent advances of computerized image analyses, several methods of sperm morphometry have been introduced [117–123]. These morphometric analysis systems provide objective assessments of individual sperm head size and shape. Sperm morphometry is now routinely used as part of the assessment of reproductive hazards to the male workers [124].

Sperm concentration, sperm morphology, and sperm head morphometry all provide indices of the integrity of spermatogenesis and spermiogenesis. Thus, the number of sperm in the ejaculate is directly correlated with the number of germ cells per gram of testis [125], while abnormal morphology is probably a result of abnormal spermiogenesis. Azoospermia is probably the most severe observation, as it is often an indication that type A spermatogonia have been lost and recovery is unlikely.

Genetic damage is difficult to detect in human sperm [126]. Epidemiological studies of large populations have demonstrated increased frequency of adverse pregnancies in women whose husbands were working in various occupations [127]. This is primarily because the chromosomes are in interphase and there is no replication and no production of proteins. Some of the methods being used to detect genetic damage with varying success are fluorescent in situ hybridization [128–130] of certain chromosomes, TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling), comet, and the sperm chromatin stability assay [131–134]. DNA adducts may also provide information about spermatogenesis at the genetic level. Several reports have shown that paternal exposure may affect pregnancy or the health of the

offspring. These data have stimulated research into the genetic stability of the sperm cell and the cause/effect relationships of damage to sperm.

7.13 ACCESSORY SEX GLANDS

Seminal plasma is not essential for fertilization; thus, the artificial insemination of sperm collected from the epididymis results in conception. On the other hand, seminal plasma contributes importantly to the normal coitus-fertilization scenario. Seminal plasma serves as a vehicle for sperm transport, a buffer from the hostile acidic vaginal environment, and an initial energy source for the sperm. Cervical mucus prevents passage of seminal plasma into the uterus. Some constituents of seminal plasma, however, are carried into the uterus to the site of fertilization by adhering to the sperm membrane.

The viability and motility of spermatozoa in seminal plasma are typically a reflection of seminal plasma quality. Alterations in sperm viability or alterations in sperm motility parameters would suggest an effect on the accessory sex glands.

Biochemical analysis of seminal plasma provides insights into the function of the accessory sex glands. Chemicals that are secreted primarily by each of the glands of this system are typically selected to serve as a marker for each respective gland. For example, the epididymis is represented by glycerylphosphorylcholine; the seminal vesicles, by fructose; and the prostate gland, by zinc. Note that this type of analysis provides only gross information on glandular function and little or no information on the other secretory constituents. Measuring volume provides additional general information on the nature of seminal plasma.

Seminal plasma may be analyzed for the presence of a toxicant or its metabolite. Heavy metals have been detected in seminal plasma using atomic absorption spectrophotometry [135], while halogenated hydrocarbons have been measured in seminal fluid by gas chromatography after extraction [135] or protein-limiting filtration [136].

A toxicant or its metabolite may act directly on accessory sex glands to alter the quality or quantity of their secretions. Alternatively, the toxicant may enter the seminal plasma [137–139] and thereby affect the sperm and the body of the female partner after intercourse or may be carried to the site of fertilization on the sperm membrane and affect the ova or conceptus.

There are few reports of toxicant effects on the accessory sex glands in humans. Ethylene dibromide (EDB) is one example of a toxicant that exerts posttesticular effects. Short-term exposure to the toxicant reduced sperm velocity and semen volume [140]. Chronic exposure decreased sperm motility and viability, decreased seminal fructose levels, and increased semen pH [140]. An EDB metabolite was present within the semen of some exposed workers [136]. Other potential toxicants that have been detected in semen include lead, cadmium, hexachlorobenzene, hexachlorocyclohexane, dieldrin, and polychlorinated biphenyls [135]. Cocaine has been shown to bind to the sperm membrane [141].

Several sperm assessment methods measure the sperm function [142] and may evaluate sperm across more than one of the subjective toxicant site divisions outlined above. The penetration of sperm through cervical mucus (or viscous fluids

stimulating cervical mucus) [143–145], the penetration of sperm into a zona-free hamster egg (sperm penetration assay [SPA]) [146], the penetration of sperm through a zona pellucida removed from immature human ova (hemi-zona assay), and the binding to hyaluronic acid [147] have been shown to evaluate different sperm functions [148,149]. With the exception of SPA, these have not been utilized in assessing reproductive toxicants in the field setting. SPA has been used with limited success [146,150].

7.14 SEXUAL FUNCTION

Human sexual function refers to the integrated activities of the testes and secondary sex glands, the endocrine control systems, and the central nervous system–based behavioral and psychological components of reproduction (libido). Erection, ejaculation, and orgasm are three distinct, independent physiological and psychodynamic events that normally occur concurrently in men. If details regarding functions or mechanisms are desired, several reviews and in-depth reports are available [151–153].

Burnett [154] recently published a review on the effects of environmental exposures on erectile function. Assessment of occupational exposure–induced anomalies of sexual function is difficult. The researcher usually must rely on the testimony and recall of the worker regarding his sexual function. This testimony may often be confounded by the bias of the individual to guard his ego or masculine image or to attribute a preexisting libido problem to exposures at work.

Burris et al. [155] reported application of a monitor (Rigiscan®) for assessing erection at home. The assessment of erectile function using the Rigiscan has been used successfully in the occupational setting in studies of the effect of bicycle saddles on bicycle patrol officers [90,156].

The assessment of ejaculate volume may provide information on the integrity of the emission phase of ejaculation. This is, of course, complicated by effects on the accessory sex glands' capacity. Thus, a semen sample of reduced volume but with a normal ratio of constituents (marker chemicals) supports a diagnosis of an emission phase defect.

The numbness or loss of feeling of the penis can be objectively measured using a biothesiometer. The equipment can easily be set up in a private room (i.e., a restroom) and the computer operator can be in an adjoining room. The study subject places his penis in a plastic trough and the computer operator sends signals to the apparatus to increase or decrease vibration to detect the level of vibration that can be sensed by the penis [90].

7.15 FUTURE ASSESSMENT METHODS

There are several new methods that may play a key role in future studies of toxicant exposures and male reproduction [157,158], especially those that detect genomic damage [159]. As new methods are added to the reproductive health profile, there are some potential limitations that need to be considered: Are methods practical in an environmental or occupational field setting (or easily preserved for later assessment)? Is there adequate statistical power with typical field study sample sizes assessing

accuracy and precision? Is there enough semen available in most specimens to analyze all of the measures (a prioritization scheme may be needed)?

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Section III

Reproduction and Development

Biological and Computational Methods