CHAPTER 25.

Disorders of reproduction

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Summary

This chapter focuses on biomarkers of reproductive health and disease that have been developed in the past 15 years. Due to the gender- and age-dependency of most of the advances in measuring reproductive health status and outcomes, these biomarkers have been categorized with respect to the unique member of the reproductive triad of interest (i.e. mother, father, conceptus). Biomarkers of female and male puberty, female reproductive function, fetal and infant development, and male reproductive function are discussed. The strengths and limitations of developing and implementing biomarkers in reproductive health studies over the past decade are explored.

Introduction

The utilization of biomarkers in reproductive and perinatal health research has greatly enhanced our understanding of these critical areas of public health. There has been increasing emphasis on these time periods in early development as vulnerable windows over the life course, during which humans are most highly susceptible to the effects of exposure to toxic agents in the environment. The periconceptional, prenatal, perinatal and peripubertal time periods are considered to be the most susceptible intervals for adverse health events (1–7). However, methodologic issues unique to this area of research render the identification of appropriate biomarkers a daunting challenge.

Reproductive epidemiology studies often consist of a triad, including the mother, father and conceptus, which constitutes the unit of both observation and analysis. The ability to obtain biomarkers for all three of these subjects varies greatly, compounded by the differing time intervals of concern for each subject in terms of biomarkers of exposure, susceptibility and effect. Other challenges include the interrelatedness of reproductive outcomes across the spectrum of time-dependent endpoints of interest and the accuracy and reliability of the markers available for evaluation. Examination of effects that occur at later time points in gestation (e.g. recognized spontaneous abortions or preterm

delivery) are restricted to those conceptions that have survived long enough to be identified for evaluation. This reinforces the urgent need to develop methodologies, including biomarkers, that enable us to examine the earliest outcomes along this spectrum (8).

Context and public health significance

In developing biomarkers that would be appropriate for use in large-scale epidemiological studies of reproductive outcomes, one must consider not only sensitivity, specificity, predictive value, within-subject reliability (low coefficients of variation) and cost, but also acceptability and ease of use by study participants (9). Several studies have reported an increase in participation rates when study subjects are taught how to collect and ship biological specimens from the privacy of their own homes, as opposed to having the samples collected in clinics or field offices (10).

In this chapter, the earlier review by Lemasters and Schulte (11) is updated, focusing on biomarkers of reproductive health and disease that have been developed in the 15 years since that publication. Due to the gender- and age-dependency of most of these advances in measuring reproductive health status and outcomes, the biomarkers have been categorized with respect to the unique member of the reproductive triad of interest (i.e. mother, father, conceptus). Detailed discussions of advances in molecular biomarker technologies to measure exposure to environmental and infectious agents in reproductive epidemiology studies (beyond the scope of this chapter) are covered in Chapters 9–13 of this text and in comprehensive reviews devoted to these topics (12–20). Likewise, readers are referred to Chapter 7 and the wealth of resources described in Perera and Herbstman (14), Burke *et al.* (21), Seminara *et al.* (22), Field & Sansone (23) and Ho & Tang (24) for more in-depth information on developments in genomic, transcriptomic, proteomic, metabolomic and epigenomic technologies to examine disease susceptibility and etiopathogenetic pathways in reproductive epidemiology research. An overview (25) describes how the combination of bioengineering and bioinformatics has evolved to help reveal integrated, dynamic molecular networks underlying complex functions in biological systems like human reproduction and early development. Also beyond the scope of this chapter, but covered well in several recent publications, are genome-wide association studies of reproductive health outcomes (26–33).

Figure 25.1 provides an illustration of the spectrum of reproductive outcomes (although not exhaustive) that are available for investigation. This figure attempts to present these topics in a chronological fashion, from the earliest sentinel of potential adverse reproductive function among males and females, to early or delayed onset of puberty, and extending to childhood cancers in their offspring that may be linked to prenatal exposures. Again, the earliest events along this chronological spectrum represent the target areas of greatest focus, as these outcomes enable the examination of a representative cohort at risk, and may serve as early sentinels of exposure to toxic agents in the environment, a major and unresolved public health concern.

Figure 25.1. Selection of reproductive outcomes available for study

Biomarkers of female and male puberty

There has been persistent, increasing concern over the past several years regarding the observation that children in the USA are entering puberty at younger ages. While advancing age at puberty may reflect inadequate nutritional or socioeconomic conditions, younger ages may be indicative of other adverse scenarios, including obesity and exposure to endocrine active compounds in the environment (34). Most expert panelists assembled by the US Environmental Protection Agency (EPA), the National Institute of Environmental Health Sciences (NIEHS) and Serono Inc. to evaluate secular trends in the timing of puberty concluded that there is sufficient evidence of earlier breast development onset and menarche in girls (35). On the other hand, almost all the panelists agreed that there is insufficient evidence regarding trends in male pubertal development.

The Tanner scales have been widely used by clinicians for several years to examine onset of puberty in girls and boys. The scales assess stages of breast development, pubic hair growth, genitalia changes, and age at menarche (36). A method of self-assessment using photographs and written descriptions of the various stages of development was developed and evaluated by researchers in different populations (37–39). A recent review of biological markers for assessing puberty status, however, indicated that many study participants are reluctant to undergo this examination by a clinician, and several studies have indicated a range of correlations between self-reported and physician Tanner scores, depending upon many factors including race/ethnicity, age and certain psychological disorders (40). Moreover, many young individuals are reluctant to perform this self-assessment, even in the privacy of their own homes; one study had a 61% response rate when participants were asked to complete the procedure at home and mail in their information (41).

There are several biomarkers currently undergoing evaluation for use in ascertaining pubertal status. Most have limited feasibility for use in large, population-based studies, as the components of interest have short serum half-lives and would require the collection of serial blood samples. These include leptin, an adipocyte hormone involved in energy homeostasis that also interacts with the reproductive axis, and Müllerian inhibiting substance (MIS), a glycoprotein hormone produced by the Sertoli cells of the male during fetal development that causes regression and atrophy of the Müllerian ducts (42–44). Leptin is a critical regulator of body fat stores, which may underlie its role as a possible biomarker of approaching puberty. Given the well-known changes in body fat mass and percent body fat associated with puberty, it is hypothesized that leptin may serve as a biomarker of peripuberty and pubertal advancements (45). In girls, serum leptin levels rise markedly as they approach puberty, and this increase is correlated with body fat mass. The levels continue to increase throughout puberty, whereas among boys, there is an initial increase during peripuberty, followed by a return to prepubertal levels as they advance through puberty (43). The ability to measure leptin in urine would greatly enhance the utility of this biomarker in studies of peripubertal events. A recent cross-sectional study of 188 children, aged 5–19 years, reported a correlation of r = 0.65 ($P < 0.01$) between serum and urinary leptin levels (46). Of note, urinary leptin levels corresponded to serum levels and patterns by gender during puberty.

The gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), and the sex steroid hormones, estrogen and testosterone, also increase in early puberty, and can be measured in urine (47,48). A small longitudinal study recently evaluated the relationship between urinary leptin and gonadotropin levels in 13 boys and seven girls over a six-month period as they were expected to approach puberty (49). Three consecutive first morning urine samples were collected each month. These results indicated significant correlations between urinary leptin and LH levels (r = 0.43, $P < 0.001$) and FSH levels (r = 0.32, $P < 0.001$). Moreover, urinary leptin levels were higher among the girls, and were increased among both girls and boys nearing puberty compared with those remaining prepubertal over the course of the study.

Both MIS and inhibins, peptides that suppress FSH levels, have been characterized as potential biomarkers of pubertal onset in males. MIS is detected at high levels during late infancy in males, then declines gradually until the presence of primary spermatocytes are detected, which appear to inhibit MIS (50). In contrast, MIS is only synthesized postnatally by granulosa cells in pubertal girls, and is measured in serum (51,52). Serum concentrations are similar in both sexes after puberty (53). Inhibin B is a gonadal polypeptide hormone that regulates, via a negative feedback loop, the synthesis and secretion of FSH (54). Similar to MIS, among males there is a peak concentration of serum inihibin B in

infancy, followed by a rapid decline until onset of puberty, when another rise in serum levels is noted. Inhibin B is also associated with FSH, LH, testosterone and testicular volume in varying patterns throughout puberty (54,55). In girls, serum inhibin B is positively correlated with age and FSH levels during childhood, and an increase during early breast development stages (56). However, because these biomarkers currently require drawing blood samples for measurement and biological variability (e.g. diurnal fluctuations) is unknown, research is needed to develop valid and sensitive urinary biomarkers that will also be feasible in terms of cost and acceptance by study participants in longitudinal studies (57).

Molecular epidemiology studies associating environmental exposures with puberty onset

The few epidemiologic studies that have associated pubertal development with exposure to endocrine disrupting chemicals (e.g. polychlorinated and polybrominated biphenyls (PCBs and PBBs) and phthalate esters) have been the topic of several recent reviews (58–60). Conflicting findings and uncertainties regarding critical windows of susceptibility and the possibility of exposure to complex mixtures of chemicals that may have antagonistic effects highlight the need for further research with objective biomarkers. A recent epidemiologic investigation of pubertal stages in nine-year-old inner-city girls in New York City was unique in associating delayed breast development with high levels of the hormonally active agents phytoestrogens and isoflavones measured in urine (61). An expert panel recently convened to review the association between endocrine-active chemicals in the environment and altered timing of pubertal onset concluded that the evidence available appears suggestive (62). Future epidemiologic research to elucidate gene-environment interactions and the molecular pathways mediating the influence of environmental exposures on pubertal development is eagerly awaited.

Biomarkers of female reproductive function

Female libido

Changes in usual patterns of sexual desire can also serve as an early sentinel for exposures that may adversely affect reproductive health. Most of the concerns regarding a relationship between exposure to endocrine active compounds and decreased libido have focused on males, although some have questioned whether this may also be a problem among women (63). The complex interactions between sex steroid hormones and the hypothalamic-pituitary-gonadal axis at varying times over the menstrual cycle also add to the challenges in measuring these hormones as biomarkers of decreased libido (64). There have been several recent studies of diminished sexual desire in females, but the majority have examined this condition among postmenopausal women (65–68). A related outcome, hypoactive sexual desire disorder (HSDD), is defined as low sexual desire accompanied by personal distress caused by this decrease in sexual desire (69,70). The prevalence of low sexual desire among younger and middle-aged women that is not ascribed to menopausal effects ranges from 24–31% (71,72). While questionnaires to ascertain HSDD have been tested for validity and reliability, a biomarker for HSDD in women remains elusive. Although there is a growing body of evidence supporting the role of testosterone and sexual desire in women, the association between decreased serum androgen levels and women reporting low libido remains unclear (73,74). The development of assays to measure testosterone levels in saliva samples would greatly facilitate the investigation into the potential relationship between exposure to endocrine active compounds in the environment and decreased libido (75,76).

Menstrual cycle characteristics

Alterations in menstrual cycle characteristics may also serve as early sentinels of exposure to potentially harmful environmental contaminants. Furthermore, changes in menstrual cycle parameters have been associated with adverse reproductive outcomes, including infertility and spontaneous abortion (77–80). One small study (n = 14) determined that urinary FSH was significantly lower in the periovulatory period in cycles that did not result in conception compared with those that did, rendering urinary FSH a potentially useful predictor of cycle fecundity.

Numerous studies have been conducted using questionnaires to obtain information on cycle length, days and severity of menstrual flow, and dysmenorrhea; however, these methods often do not yield valid information. One study described the use of urinary biomarkers to determine sex steroid hormone levels throughout the menstrual cycle among healthy premenopausal women (n = 403) (81). The women were required to collect and freeze daily morning urine samples that

were analysed for pregnanediol-3-glucuronide, estrone sulfate and estrone glucuronide (combined and referred to as E1C) by enzyme-linked immunoassay. Using computer-generated algorithms to define menstrual cycle events, the researchers were able to utilize the hormone data to describe menstrual cycle length, the length of both the follicular and luteal phases, as well as occurrence and timing of ovulation.

Using the same sampling frame as the study above, the Kaiser Permanente Medical Care Program in California evaluated variability in estrogen and progesterone metabolites according to these menstrual cycle characteristics, as well as demographic variables and reproductive history (82). With an average length of participation of 141 days, urine samples were collected on over 95% of the study days in this sample of mostly white, highly-educated women. They reported urinary estrogen metabolite levels 10–13% higher in the baseline interval (days 1–5) and during the follicular phase among women who experienced shorter menstrual cycles. There was also an association between increased urinary progesterone metabolites and a longer luteal phase. Associations between estrogen and progesterone metabolites and race/ethnicity, prior reproductive experiences, age, BMI and educational level were also noted.

A large study was conducted in 1989–1991 to determine the relationship between occupational exposures among women employed in the semiconductor industry and fertility and early pregnancy losses (83). Investigators evaluated the influence of demographic and lifestyle factors on menstrual cycle characteristics among 309 women from this cohort. Duration of follicular and luteal phase segments, and occurrence and timing of ovulation were determined using urinary estrogen and progesterone metabolite levels that were analysed in daily morning urine samples. These efforts confirmed earlier reports that menstrual cycle characteristics vary by age, race/ethnicity and lifestyle factors (e.g. alcohol consumption) (84–86).

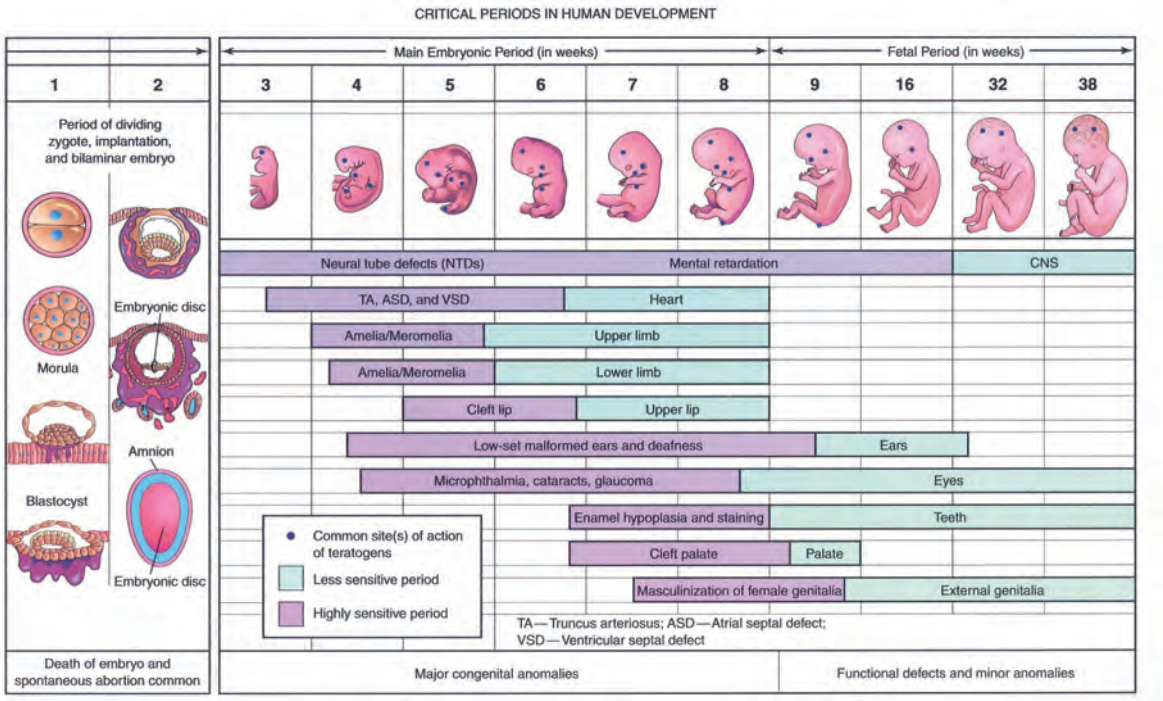
Taken together, these studies provide evidence that urinary biomarkers can be used in large, population-based studies of menstrual cycle function, as well as in selected fertility and pregnancy outcomes. However, it must be noted that the majority of these protocols required daily specimen collection over varying lengths of time. While compliance rates with urine specimen collection in prospective pregnancy studies is generally quite high (8), it must also be noted that these studies typically involve women (or couples) who are planning to conceive. There may therefore be considerable variability with compliance, depending upon the goal of the study and the pregnancy intentions of the sample.

Additional biomarkers that would be feasible for use in large, population-based studies focusing on the detection of ovulation include cervical mucus monitoring, which has been available and used for several years, and salivary sex steroid hormones levels. However, studies examining the validity and feasibility of analysing salivary samples for various biomarkers of reproductive function have included relatively small sample sizes, and reported considerable intra- as well as interindividual variability for the sex steroid hormones (87–91).

Infertility/early pregnancy loss

The only approach that allows researchers to distinguish between women having infertility and those experiencing subclinical or early pregnancy loss is the prospective pregnancy study design. Though beyond the scope of this chapter because of limited application in large-scale epidemiologic research, diagnostic advances in assisted reproductive technology (ART) have shed light on this distinction, and readers are referred to several recent and thorough reviews (92–94). As illustrated in Figure 25.2, there is increased emphasis on the periconceptional environment that may impact fertilization as well as implantation. Scientists have made great strides in the ability to detect early pregnancies once implantation takes place. The measurement of urinary human chorionic gonadotropin (hCG) as a marker of early pregnancy loss has been used since the late 1980s (95–97). The algorithm for determining the level of hCG that exceeds normal background levels and indicates that conception has occurred was developed by examining hCG levels throughout the menstrual cycle in women who had been surgically sterilized (96). Concerns were later raised suggesting that urinary hCG alone may not be a sensitive indicator of early pregnancy among those conceptions terminating in subclinical losses (98–100). However, when patterns of serum and urinary hCG levels were compared between successful pregnancies and those terminating in early pregnancy loss and clinical spontaneous abortion, there were no differences, thus validating the solitary measure of urinary hCG as a biomarker of pregnancies around the time of implantation (101).

Figure 25.2. The sensitive periods in human development (230). Copyright Elsevier (1998).



A prospective, longitudinal study of early pregnancy loss (conceptions ending within five weeks of ovulation) among indigenous women of rural Bolivia correlated salivary progesterone measurements with urinary hCG measurements (102). Among the 191 study women who were eligible (taking no active steps to either prevent or achieve conception) and visited a clinic every other day (to collect samples and record the first day of menstrual bleeding), eight early pregnancy losses were detected and 32 pregnancies were sustained past the five-week cut point. Overcoming some of the limitations of salivary progesterone measurements described above, the investigators were able to detect ovulation as a sudden, steep rise in salivary progesterone, and they reported a significant association between elevated follicular phase (pre-ovulatory) progesterone and subsequent early pregnancy loss.

The development of fertility monitors and sensitive urinary hCG assays that can easily be used by women in the home to detect early pregnancy has greatly enhanced the ability to examine fecundability, infertility, time-to-pregnancy and early pregnancy loss (103,104). The Oxford Conception Study is a randomized clinical trial to determine if knowledge of the timing of peak fertility increases conception rates among couples trying to achieve pregnancy (103). The study is using the Clearblue® Easy fertility monitor, which measures levels of urinary estrone-3-glucuronide (E3G) and LH. The monitor screen displays bars to indicate high and peak (which displays an egg symbol) fertile days in the cycle. There are two intervention arms to the study: one third of women receive feedback only on the early fertile time, defined as the first rise in E3G until the LH surge is detected, and another one third receive information only about the late fertile time, or the onset of the LH surge and the subsequent two days. The control group does not receive any fertility monitor feedback concerning fertile windows. This same fertility monitor is also currently being used in the Longitudinal Investigation of Fertility and the Environment (LIFE) study, a prospective examination of the impact of environmental exposures and lifestyle factors on fecundability and fertility, which will be described later.

There are additional advantages to using the fertility monitors. Women are requested to collect first morning urine samples beginning on day six of their cycle, and continue for 10 or 20 days depending upon their cycle length; thus the need for daily urine sample collection is reduced somewhat. Moreover, the monitors store data on the estrogen and progesterone metabolite levels, which may be downloaded using a data card and processed for data analysis. These hormone

data are also used to generate graphs for study participants, which provide a clear illustration of their fertile windows during each cycle, and reinforce the importance of engaging in sexual intercourse on those days to increase likelihood of conception.

The utilization of fertility monitors and home pregnancy test kits as described above has greatly enhanced the information that can be obtained in prospective pregnancy studies. However, the home pregnancy tests cannot detect fertilization, and only begin to measure hCG around the time of implantation. Thus, the interval between fertilization and implantation remains a 'black box' in the investigation of factors affecting fertility. There have been some attempts to measure a substance known as early pregnancy factor (EPF) (105–107). EPF is believed to be a substance secreted by the ovary in response to a trigger from the zygote (107). This 'ovum factor' is secreted at the time that the ovum is fertilized, and can be measured in the maternal serum within 2–6 days post ovulation (106). Despite the fact that several earlier studies demonstrated the ability of EPF to detect fertilization in humans before implantation, this biomarker has not been evaluated for use in prospective studies of fecundability and fertility. Again, the need to draw blood samples during each menstrual cycle to detect EPF, renders this less than optimal in large-scale population-based studies, but a recent study reported the presence of EPF in the cervical mucus of pregnant women (108). Mean EPF activity, measured in rosette inhibition titres (RIT), was significantly higher among 53 pregnant women during their first trimester of gestation, and seven women in the second trimester, compared with 25 non-pregnant women (6.58, 5.71, and 3.44 RTI, respectively ($P < 0.001$). Moreover, there was a significant correlation between serum and cervical mucus RTI values, r = 0.611 ($P < 0.0005$). Additional research on EPF activity in cervical mucus around the time of ovulation is needed to determine the feasibility of this biomarker in identifying fertilization and preimplantation events in fecundability and fertility studies.

The challenges inherent in establishing and maintaining prospective pregnancy study cohorts underscore the importance of developing biomarkers that are sensitive, specific and cost-effective but also acceptable and relatively easy to use. The LIFE study, designed to examine the relationship between environmental and lifestyle factors and fecundability and fertility, is funded by the US National Institute for Child Health and Human Development and is currently enrolling couples in Texas and Michigan (http://www.lifestudy.us). This study methodology is the only approach that allows for ascertainment and examination of the early critical events in the reproductive process in humans (i.e. to distinguish between failures of fertilization versus subclinical pregnancy losses), and hopefully soon, implantation failures versus these other two outcomes. The LIFE study recruitment efforts have determined that willingness to comply with protocol requirements, including collection and testing of biological samples and completion of diaries during the interval of attempting to conceive, as well as changes in pregnancy intentions due to varied life events while enrolled in the study, illustrate the need both for the development of sensitive and acceptable biomarkers, as well as very large sampling frames in population-based prospective pregnancy studies.

Biomarkers of fetal and infant development

Adverse fetal or infant outcomes

The major focus of this review is the identification of new biomarkers of reproductive health that can be employed in large-scale epidemiologic studies of reproduction. Although the many promising biomarkers developed recently for use in clinical obstetrics and reproductive endocrinology are beyond the scope of this chapter, readers are referred to in-depth discussions of advances in the detection of pregnancy complications (e.g. pre-eclampsia and intrauterine growth retardation) and other adverse prenatal events provided elsewhere (109–112). Several resources are also available for updates on screening and diagnostic tests for aneuploidy, including Down syndrome (113–117), other congenital anomalies (118), fetal lung maturation (119) and haematologic disorders and complications (120) during gestation. Epidemiologic studies of perinatal outcomes and long-term health in children conceived by ARTs have been an important source of prospective and high quality data. Outcomes following the more recently developed ARTs (e.g. intracytoplasmic sperm injection) are currently being compared with outcomes following the more established technologies, such as *in vitro* fertilization (121–123).

There have been recent advances in the utilization of dried blood spots (DBS) that hold great promise for large-scale epidemiologic studies aiming to identify valuable biomarkers of

susceptibility, exposure and effect. Blood spots, obtained via heel stick, are collected within 24–48 hours after birth on nearly all newborns in the USA. The spots are stored on special filter paper Guthrie cards and used by states in screening programmes to diagnose a variety of disorders among newborns. The number of different disorders assessed in the screening programmes is determined by each state's department of health. There has been a surge of interest recently in the analyses of DBS to investigate diverse disorders in large population-based studies, including environmental exposures and their relationship to congenital anomalies and developmental disorders, the prevalence of infectious diseases among newborns, and genetic disorders and potential biomarkers of susceptibility (124–129).

A recent meeting discussed the issues and approaches to using DBS in studies investigating environmental exposures and infant health outcomes (130). Preliminary work has been conducted to determine the feasibility of analysing DBS for several exposures of concern, including persistent bioaccumulative toxics (PBTs is the term used by the US EPA, whereas the United Nations Environment Programme uses the term persistent organic pollutants, or POPs), metals, infectious agents, immune factors and genetic disorders. The scientists concluded that DBS represent a very valuable source of biomarkers of exposure, effect and susceptibility, but there are limitations that must be resolved before they can be used to the maximum potential. The limitations include inadequate sample volume, as many laboratory techniques to measure environmental contaminants require relatively large volumes; development of reference values for elements with large variability in whole blood matrices; and issues related to stability, recoverability, half-life and storage over time. Great care must be taken in the collection, drying, storage and transport of the spots if they are to be of future use in research studies (131). There is also the critical need to develop policies regarding the ethics and human subjects research challenges presented by these specimens (132,133).

Molecular epidemiology studies associating environmental exposures with fetal and infant development

An extensive review of the epidemiologic literature (including original studies, expert panel reports, meta-analyses, and pooled analyses) published between 1970 and 2006 examined adverse reproductive and developmental outcomes in relation to preconceptual and prenatal exposures to environmental compounds (18). The pregnancy outcomes examined included fetal loss, intrauterine growth retardation, preterm birth, birth weight, congenital anomalies and childhood cancers among others. The environmental exposures included metals, pesticides and hormonally active agents (e.g. methyl mercury and PCBs). The question of whether *in utero* exposure of male offspring to hormonally active agents in the environment (e.g. the plasticisers, phthalates) (134) increases their risk of testicular dysgenesis syndrome (TDS) (i.e. impaired spermatogenesis, hypospadias, cryptorchidism and testicular cancer) in a manner similar to *in utero* exposure to diethylstilbestrol (DES), was evaluated in a recent meta-analysis (135). Although the meta-analysis confirmed the association of DES with TDS, there is as yet no compelling evidence that any other hormonally active agents increase the risk of TDS.

An enormous amount of valuable information on the reproductive and developmental effects of the atomic bombing of Hiroshima and Nagasaki has been compiled and summarized (136). The unique and precedent-setting contributions to the field of reproductive epidemiology, as a result of the investigators' careful and extensive use of biomarkers throughout the extended course of this research, are remarkable. Recent studies reporting increased risks of preterm birth, low birth weight, and small for gestational age among the births to female survivors of childhood cancer further illustrate the advantages of accurate, carefully documented biomarkers in revealing the reproductive health effects of exposures at time points early in the mother's development (137,138).

Gene–environment interaction studies in pregnancy outcomes

Molecular epidemiology studies examining the influence of gene-environment interactions on reproductive health have focused most often on the relatively common birth defects, such as neural tube defects, oral clefts, hypospadias and gastroschisis (139–147). Several these studies have linked either folate metabolism genes with maternal nutritional factors (139–141) or metabolic/detoxification pathway genes with maternal smoking (143,144,146). Maternal exposure has been assessed by questionnaire or interview (without biomarkers) and genotyping has been performed on DNA from the mother (146), infant (142,145,147), or rarely, the entire triad including the father (143,144).

Researchers have emphasized the utility of examining Mendelian randomization, or the random transmission of genes that occurs between parent and offspring, in drawing inferences from studies that examine the developmental health effects of *in utero* exposures in combination with candidate susceptibility genes (148). Studies that genotype each member of the triad separately may confer an important advantage to the interpretation of study results. Specifically, if the genetic variant under study either influences exposure to the etiologic factor of interest or modifies the exposure–disease relationship, then the expected relative risk for the adverse pregnancy outcome would be farthest from the null when presence of the risk gene is measured in mothers' DNA, closer to the null when the presence of the risk gene is measured in fathers' DNA, and somewhere in-between when presence of the risk gene is measured in infants' DNA.

Research interest in the interactions between metabolic/detoxification genes and maternal/*in utero* exposure to environmental agents has begun to extend across the spectrum of pregnancy outcomes including preterm delivery (149), infant birth weight (150), pervasive developmental disorders (e.g. autism) (151) and childhood-onset attention deficit disorder (152). These studies further illustrate the methodologic challenges often confronted by molecular epidemiologists: constraints on the study design (e.g. statistical power issues and the need to rely on case-control or case-only designs); source populations of varying race, ethnicity and genetic backgrounds (e.g. case and control groups may not be comparable); participant recruitment (e.g. requiring consent for sensitive, invasive or complicated sample collection procedures); exposure assessment (that may include idiosyncratic sample collection, processing and storage requirements and expensive analytical techniques); and data analysis strategies that must take into account important interrelationships (e.g. confounding and effect-measure modification) across large numbers of measured independent and dependent variables.

Childhood cancer

The extent to which the etiology of childhood cancer involves *in utero* or preconceptional parental exposure to environmental agents remains a major public health concern and an important research question for both reproductive and cancer epidemiologists. Biomarkers of childhood cancer and a review of the epidemiologic investigations that have incorporated molecular markers of exposure and susceptibility are discussed in detail in Chapter 26. A recent comprehensive review of pesticides and childhood cancer (153) is discussed only briefly here to emphasize the critical need for improvements in exposure assessment (e.g. objective biomarkers of exposure to environmental chemicals) and for the detection of specific gene–environment interactions that are likely contributors to the complex etiology of childhood cancer. Among the 77 studies included in the review (153), parental exposure to pesticides (including herbicides and insecticides) was measured indirectly (e.g. by proximity of residential address to chemical production plants or by classification of usual occupation on birth certificate) by responses to interviews or self-administered questionnaires, review of employment records, or by environmental monitoring techniques. None of the studies had measured exposure to pesticides using biological samples (e.g. urine or blood). This is understandable given that over time, study participants may have been exposed to several different compounds (either one at a time or in complex mixtures), that many pesticides have short half-lives in the biological samples commonly used for analysis, and that the limits of detection for many of the standard chemical analyses are relatively high.

Transgenerational health effects and epigenetic mechanisms

Several recent studies have advanced yet another challenging but essential area of research to determine adverse reproductive effects of environmental exposures—the conduct of second-generation studies in humans. The critical importance of this effort is illustrated by the studies of the offspring of women who took DES during pregnancy to prevent spontaneous abortions. Earlier animal studies suggested that the carcinogenic effects of prenatal DES exposure may be transgenerational, reporting an increase in reproductive tract tumours among the offspring of mice with prenatal exposure to DES (154–156). After noting the occurrence of hypospadias among two boys born to mothers who had been exposed to DES prenatally, researchers in the Netherlands conducted a cohort study among women experiencing fertility problems to examine this association (157). Among 205 women who reported having been exposed to DES *in utero*, four gave birth to sons with hypospadias, compared with eight cases reported

among the 8729 sons born to non-exposed women (prevalence ratio = 21.3; 95% CI = 6.5–70.1). This finding generated several studies in more representative populations, all indicating an increase in hypospadias among sons of prenatally exposed DES mothers. However, the magnitude was much lower, with prevalence ratios of 5.0 (95% CI = 1.2–16.8) among a French population and 1.7 (95% CI = 0.4–6.8) among women in the USA, and a case–control study of both maternal and paternal *in utero* DES exposures yielded an adjusted odds ratio (OR) of 4.9 (95% CI = 1.1–22.3) for maternal exposures, but no increase among males whose fathers were prenatally exposed to DES (OR = 0.9; 95% CI = 0.1–6.7) (158–160). In addition, there were case reports of other congenital anomalies among second-generation offspring of DES-exposed women, including limb reduction defects, deafness and ovarian carcinoma (161,162).

There have been several recent reports on approaches to examine the transgenerational adverse reproductive effects of *in utero* DES exposure in animal models. Experiments in mice exposed to DES within 1–5 days after birth have supported the hypothesis that epigenetic dysregulation (e.g. hypomethylation of multiple CpG sites of the proto-oncogene *c-fos*) could be a causal mechanism underlying the adverse effects of DES on uterine tissue (163). As described in greater detail in Chapter 26 and recent reviews (164,165) of the 'developmental origins' or Barker hypothesis (166–168) (i.e. that environmental exposures during the earliest and most plastic stages of human development may be among the most significant causal factors underlying many chronic diseases in children and adults), epigenetic mechanisms regulate gene expression through DNA methylation, histone modification of chromatin structure, and autoregulatory DNA binding proteins.

Although epigenetic mechanisms can cause phenotypic discordance between monozygotic twins, epigenetic influences can also be inherited (e.g. an imprinted gene in which the only allele expressed in the offspring is the one inherited from either the mother or the father, never both). Epigenetic alterations can occur at the level of transcription, translation or post-translation, and appear to mediate the development of adverse reproductive health effects following experimental exposure to several hormonally active environmental toxicants, at least in animal models (e.g. dioxins and PBBs) (169). In rodents, for example, *in utero* exposure to the endocrine-active compounds bisphenol A (170) and vinclozolin (171) produced epigenetically-mediated changes in coat colour and in reproductive organs including testicular defects and prostate tumours, respectively. In the latter study of rats, the epigenetic changes and adverse reproductive health effects appeared to be transmitted through a paternal allele in three consecutive generations (i.e. transgenerational), despite the lack of any vinclozolin exposure beyond the first generation of pups.

As is so often the case for findings from novel and intriguing animal experiments, they await confirmation in other experiments, since replication is the hallmark of good science. The validity and reliability (e.g. coefficient of variation) of the intricate molecular techniques to measure epigenetic mechanisms in different human biological matrices with varying amounts of each individual sample are highly uncertain at this time, and gold standard methodologies have yet to emerge (24). In humans, molecular epidemiology studies able to examine biomarkers of epigenetic mechanisms and reveal the ways in which such mechanisms mediate the relation between exposure to environmental chemicals and reproductive outcomes may lag only a few years behind the ground-breaking studies in animal models. However, it may take many years before molecular epidemiologists can measure epigenetic markers and their ultimate effects on reproductive health across multiple generations. There is hope that answers will come from the National Children's Study (http://www.nationalchildrensstudy.gov), which plans to examine mothers and fathers before and during pregnancy, and to follow their children for decades thereafter (172).

Biomarkers of male reproductive function

Male libido

Biomarkers of male reproductive function have been reviewed (173), as well as a comprehensive and in-depth look at the current array of diagnostic tests available for male sexual dysfunction (174). Libido is the biological need for sexual activity (i.e. the sex drive), and male sexual desire is regulated by past sexual activity, psychosocial factors, activation of brain and spinal cord dopamine receptors, and gonadal hormones. Little is known of the physiologic basis of libido, and assessments of libido, erection, ejaculation, orgasm and detumescence would be difficult to make in large-scale epidemiologic studies. There are electronic devices adapted for home use that monitor nocturnal penile tumescence (the penile erections that occur

spontaneously during rapid eye movement stages of sleep) (174,175). Though relatively little is known about the effect of occupational or environmental exposures on sexual desire in men, it has been suggested that lead, carbon disulfide, stilbene or cadmium exposure may have adverse effects (176). The few studies that have associated erectile dysfunction with exposure to hazardous environmental and occupational chemicals, and the fewer still that have incorporated biomarkers of exposure, have been carefully reviewed (175). Clearly, male libido and erectile dysfunction are important reproductive health outcomes requiring further epidemiologic research and biomarker development.

Hormones

Chemical analyses for male hormones are usually performed on serum or urine samples, and the latter are readily available for use in large-scale epidemiologic studies. Abnormal levels of male hormones in serum or urine are indicators of problems in the hypothalamic-pituitary-gonadal axis that underlie abnormalities observed in semen analysis (e.g. azoospermia and oligospermia) (177). As a modestly invasive procedure, requiring relatively little formal training, blood collection in males is fairly well tolerated and inexpensive. The National Institute for Occupational Safety and Health recommends a profile including FSH, LH, testosterone and prolactin to evaluate endocrine dysfunction in the male. Although LH and FSH can be measured in urine, prolactin is currently measured only in serum (173).

As high rates of refusal among study candidates has remained a major barrier to the assessment of semen quality in population-based epidemiologic investigations, the identification of alternative reliable biomarkers is a pressing need (20). In a recent comparison with the gold standard measurement of sperm concentration in semen, serum levels of inhibin B, the peptide hormone produced in Sertoli cells, looked promising as a potential surrogate biomarker for large-scale epidemiologic research (178). Although serum FSH has also been used as a surrogate biomarker for semen quality, FSH levels may be less desirable on the biological grounds that they are affected by gonadotropin releasing hormone, estradiol and testosterone, and unlike inhibin B, FSH is not produced in the testes (178). In the future, serum biomarkers for other male hormones, such as activin and follistatin, may be explored for their utility in epidemiologic studies of male reproductive function (173).

Molecular epidemiology studies associating environmental exposures with male hormone levels

A recent, unique molecular epidemiology study compared serum prolactin and inhibin B levels in male welders with corresponding levels in an age-matched comparison group. The investigators reported significantly positive associations after adjusting for smoking and alcohol consumption (179). Whole blood manganese concentration was also positively associated with serum prolactin level. As the higher serum inhibin B concentrations in welders compared with the referent group were contrary to expectation, the findings of this novel study await confirmation (179). Several recent epidemiologic studies have examined the effects of exposure to environmental agents on both male hormones and semen quality, allowing for a more thorough assessment of the potentially complex environmental effects on male reproduction (180–182).

Semen characteristics

Despite conflicting reports and substantial geographic variation, the question of whether declines in semen quality and sperm counts over the past several decades have resulted from exposure to post-industrial age environmental toxicants remains a major unresolved public health concern. While the number of reports of declining sperm counts continues to grow, there is as yet no compelling evidence of decreased fertility in the human populations studied (173,183). The assessment of reproductive function in males usually begins with semen analysis (177). In addition to the challenges of recruiting participants willing to submit semen samples for large-scale epidemiologic studies, the samples must be collected in appropriate containers, analysed within one hour of collection, and kept warm during transportation (177).

The systematic analysis of semen includes macroscopic and microscopic evaluations and chemical assays. Samples are examined for liquefaction, viscosity, colour, pH (normal = 7.2–7.8), volume (normal = 2–5 millilitres), sperm concentration (normal = 20–50 million per millilitre), morphology (e.g. size of the acrosomal cap and length of the tail, normal ≥ 50% of sperm have a typical acrosomal shape and size and a tail around 45 µm in length), motility and velocity/progression (normal ≥ 50% of observed sperm are motile and move forward rapidly in a straight line with little lateral movement), agglutination (clumping) and the presence of other cellular elements

(e.g. immature germ cells and leukocytes) (20,177).

Although automated semen analysis systems have been developed, visualization and interpretation of important subtleties in human sperm are difficult. Evaluation by manual methods remains the standard practice (177). Nevertheless, researchers should be aware that evaluations of sperm concentration and motility have demonstrated acceptable interlaboratory reliability and low coefficients of variation, but the assessment of other sperm characteristics (e.g. progression) has been more difficult to standardise (184). Semen contains a vast array of antigenically diverse proteins, including those carried on the surface of sperm, and the largely unknown influence of specific male reproductive proteins on human conception and pregnancy outcomes will be an important focus for future molecular epidemiology research (185).

Although semen has been used for biomonitoring of exposure to metals and xenobiotics (20), concerns have been raised that routine semen analysis may be an insensitive measure of many important reproductive health effects resulting from environmental exposures (19,20). A more comprehensive approach includes assessments for cytogenetic sperm abnormalities and DNA damage (19,20). The fluorescent *in situ* hybridization (FISH) technique has been widely used to detect aneuploidy, chromosomal breaks, and rearrangements in sperm cells. Although the FISH technique is efficient for large-scale use, an immense number of each subject's sperm cells must be evaluated (up to 10 000) (20).

Sperm chromatin is extremely compact and stable relative to chromatin from somatic cells (20), and this property has led to the development of novel biomarkers of sperm DNA integrity. Damage to sperm from reactive oxygen species (e.g. oxygen ions, free radicals and peroxides) has been shown to contribute to reductions in male fertility (20,186). In fact, the production of free radicals due to oxidative stress was first reported in sperm cells (186). Oxidative DNA damage refers to the functional or structural alteration of DNA that contributes to many degenerative diseases of aging including cancer (20). Oxidative stress to sperm DNA integrity can arise endogenously or from exposure to environmental toxicants including xenobiotics (19). Oxidative stress leads to impaired sperm motility, reduced fertilization, and DNA damage.

Although there are over 30 assays of oxidative stress available for sperm assessment, the cost and complexity, combined with difficulties in standardization across laboratories, limit their use especially for large-scale epidemiologic research (186). The level of the oxidative DNA adduct 8-hydroxy-2'-deoxyguanosine (8-OHdG) in sperm is considered a sensitive and precise biomarker of oxidative DNA damage (19,20). High levels of 8-OHdG are positively correlated with abnormal sperm morphology and negatively correlated with sperm concentration, number and motility (19). In addition to the 8-OHdG assay, a variety of methods to measure sperm DNA strand breaks have developed over the past 25 years, leading to the four major tests of sperm DNA fragmentation in current use today (mostly in ART laboratories): the Comet, Tunel, sperm chromatin structure assay (SCSA) and the acridine orange test (AOT) (186,187). The Comet, Tunel, and AOT techniques use light microscopy; however, the Tunel technique can also be performed with flow cytometry. The SCSA method requires flow cytometry (187). Challenges remain in standardization, establishing thresholds and reference ranges, and achieving acceptable levels of interlaboratory reliability for these assays (187).

Molecular epidemiology studies associating environmental exposures with semen quality

The literature on epidemiologic studies relating semen quality to exposure to pesticides, and other endocrine disrupting chemicals has been thoroughly reviewed (188–190). The overwhelming consensus is that the studies have varied considerably in methods, exposures and outcomes, and the results have been equivocal. The need for further research in this area is compelling. Several molecular epidemiologic studies have begun to incorporate biomarkers of DNA damage to sperm (e.g. SCSA and the DNA fragmentation index) as more sensitive measures examine the effects of exposure to potentially toxic environmental compounds (191–194). Molecular epidemiology studies have also begun to examine the influence of gene–environment interactions on sperm DNA integrity (195,196).

The evidence for male-mediated reproductive and developmental toxicity has been reviewed (197). There is some evidence that irradiation and exposure to certain chemical compounds can be genotoxic to sperm in experimental animals, leading to the development of malformations and tumours in their offspring. On the other hand, paternal exposure to low levels of non-mutagenic compounds, such

as lead, has altered learning and mating behaviour in offspring, but has not led to obvious malformations or tumours (197). Evidence of male-mediated reproductive and developmental effects in humans has derived mostly from studies of children born to men exposed to environmental toxicants (e.g. methyl mercury, anaesthetic gases, lead, solvents and pesticides) through their occupations. These studies have been limited by the lack of objective and precise measures of exposure and by the failure to adequately account for exposures in the mother. The mechanisms proposed for male-mediated reproductive toxicity in humans involve direct effects from contaminated seminal fluid, as well as both genetic and epigenetic pathways. These pathways could involve germ cell mutation, sperm DNA instability, suppression of germ cell apoptosis, or interference with genomic imprinting (197). As available animal models may have reproductive systems and exposure regimens that poorly approximate conditions in humans, evidence for or against male-mediated reproductive toxicity and the hypothesized underlying molecular mechanisms will largely depend on the validity and precision of future epidemiologic investigations.

Strengths, limitations and lessons learned

There have been tremendous strides in the development and implementation of biomarkers in reproductive health studies over the past decade. These advances have greatly enhanced our ability to explore potential etiologies of and increased susceptibility to adverse reproductive outcomes. In addition, these new techniques have enabled us to identify precursor events and more subtle manifestations of these disorders, as well as to elucidate underlying mechanisms. This is of great benefit to public health, as we now are better positioned to identify early sentinels of exposure to toxicants and to intervene to reduce human exposures.

The capability of measuring the dose of exposure to a toxic agent in individuals greatly reduces exposure misclassification (e.g. recall bias), especially notable in scenarios where the individuals have little knowledge of their exposure. Biomarkers of exposure also enable more precise determinations of dose–response relationships, which can vary across age and gender. The recent focus on critical windows of susceptibility has also affected the risk assessment process, as evidenced by the use of age-dependent adjustment factors (ADAFs) by the EPA in risk assessment regarding early life exposures to carcinogens (198). For ages 0–2 years, the ADAF is 10, indicating a 10-fold increase in carcinogenic potency during this period. For ages 2–16 years, the ADAF is 3, and for ages ≥ 16 years, the ADAF decreases to 1. At present, there is no ADAF for the prenatal period, which is a limitation.

Studies in reproductive epidemiology that incorporate biochemical markers of exposure are only just beginning to address the "windows of susceptibility" issue. It is important to consider the independent and joint influences of age at initial exposure and intensity of exposure, as an environmental agent may have irreversible, long-term reproductive health effects in addition to acute or immediate effects. Perhaps because the timing of exposure is an emergent area of inquiry for epidemiologic studies of environmental chemicals, this issue is not without controversy. Recent commentaries (199,200) following our report of a significant association between infant birth weight and mother's age at initial exposure to PBBs, independent of the association with maternal serum PBB levels (201), highlight the need to consider the time-dependency of maternal exposures and the potential for differential effects on pregnancy outcomes. For example, the effects of *in utero* exposure to a biological agent that remains in maternal circulation (due either to a single exposure event or long-standing cumulative exposure) during critical periods of embryogenesis and organogenesis may be very different from *in utero* effects mediated by the mother's initial exposure to the agent during critical periods of her own childhood and reproductive development (202). Such complexities pose a significant challenge to occupational and environmental epidemiology, as well as to molecular epidemiology (203). As researchers further explore the underlying biological relationships among the growing number of measurable biomarkers, they must take care to operationally define all relevant variables, allow for time-dependencies, and be prepared to reach beyond conventional statistical modeling strategies that may be oversimplified or poorly specified (204,205).

At the same time, epidemiologists have become more alert to the periods of heightened susceptibility during early human development; there is growing awareness of the uncertainties and complexities relating to the toxicokinetics of different environmental chemicals. There is greater appreciation that nonlinear dynamics may be involved and that many interacting factors can influence the rates of uptake, biotransformation, metabolism and elimination. An especially important development is the

recognition among toxicologists that the methodologic challenges to valid exposure assessment are not unique to epidemiologic research. As underscored in a recent thought-provoking review (206), these challenges extend to experimental animal models as well. There is room for substantial improvement in biomonitoring that incorporates sensitive, repeated measures to better reveal biological variability both within and between subjects (whether human or animal).

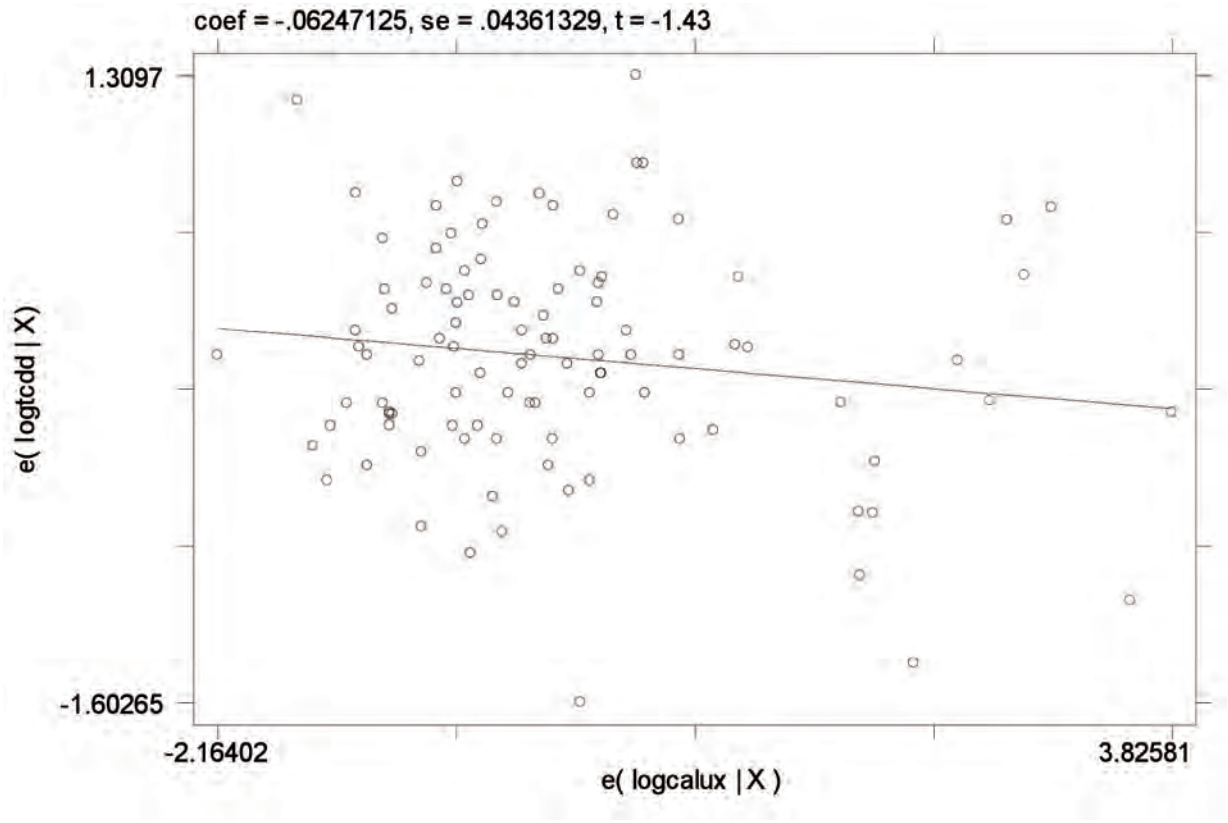
Accurate exposure assessment for some of the most toxic environmental chemicals (e.g. dioxins and the most toxic congener, 2,3,7,9 tetrachlorodibenzo-p-dioxin (TCDD)), requires potentially uncomfortable or invasive specimen collection and highly expensive, technically complicated sample preparation and analysis (207,208). This may partially explain why, for example, specific reproductive health effects of dioxin congeners, including TCDD, have been studied extensively with biomarkers in animal models, but infrequently in population-based epidemiologic studies (209). The rarity of many specific adverse reproductive health outcomes in the general population (e.g. narrowing the broad class of reportable birth defects to diagnostic categories, such as neural tube defects, or to single, clinically-defined entities, such as spina bifida) has made conclusive findings difficult to compile from the few cohorts of highly exposed individuals that have been tested for serum TCDD levels and closely monitored for adverse reproductive outcomes (209). The expected number of cases of many of the single, well-defined reproductive health outcomes of interest in these exposed cohorts is relatively small. Results from well-designed epidemiologic studies in larger populations of individuals that are expected to have a gradient of dioxin exposure (due to the environmentally ubiquitous and persistent nature of these compounds) are eagerly awaited despite the methodologic and political challenges (210–212). One strategy that has been proposed for biomarker studies of TCDD and other environmental chemicals that require either relatively large aliquots for each individual sample, expensive analytical methodologies, or both, is to design statistically powerful and cost-conserving protocols for the pooling of individual blood or serum samples (213–218).

The ability to utilize easily obtained biological specimens (e.g. urine samples and buccal swabs), rather than relying on collection of blood samples (often serially), will greatly improve the acceptability of biomarker studies in large population-based studies. A major limitation of biomarker studies is the reluctance of study participants to undergo serial blood draws, but as discussed above, there is considerable research focused on the development of alternative biological media that would be of huge benefit for researchers in this area. Use of home-based collection and storage protocols also improves compliance and reduces the costs associated with the transport and processing of the samples.

Despite the allure of inexpensive, high-throughput technologies, the appropriate application of biomarker assays to measure exposure, susceptibility, and reproductive health effects in large-scale epidemiologic research will require painstaking validation, comprehensive quality control procedures, and active participation of collaborating laboratories in regular programmes of proficiency testing. Detailed results from a comprehensive quality control programme should be thoroughly reviewed before selecting a collaborating laboratory. If at all possible, studies applying novel biomarkers or high-throughput technologies should incorporate a validation study comparing results using the new test (for at least a reasonably large random sample of participants) with results using a suitable gold standard methodology.

A validation study of a lower cost screening test for dioxin-like compounds in serum (i.e. the chemically activated luciferase reporter gene expression (CALUX) assay) in a case–control study of neural tube defects in the children of US veterans of the Viet Nam War provides a striking example (219). Of interest was the potential use of the CALUX assay to reduce costs in large-scale molecular epidemiology studies that seek to examine the association between parental exposure to dioxin congeners, especially TCDD, and adverse pregnancy outcomes. To assess the validity of the CALUX assay, results were compared with results from the gold standard method of dioxin analysis—high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). Figure 25.3 shows a lack of correlation (and in the negative direction) between the CALUX results for dioxin-like activity in serum (in TEQs) and the serum TCDD levels measured by the gold standard, HRGC/HRMS, on paired serum samples. A recent epidemiologic study of the Seveso, Italy cohort of residents exposed to TCDD in 1976 (from an explosion in a plant that manufactured 2,4,5-trichlorophenol) reported a similar lack of correlation in results from serum analyses comparing the CALUX assay with the gold standard, HRGC/HRMS (220). Had

Figure 25.3. Correlation between log-transformed serum values of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), measured by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS), and serum values of dioxin-like activity measured by the chemically activated luciferase gene expression (CALUX) assay



Y axis = log TCDD values; X axis = log CALUX values; P=0.389.

the validity of this CALUX assay been assumed (e.g. on the basis of reports of its validity in matrices other than human blood (221)), the significant three-fold association between paternal serum TCDD level (measured by the gold standard methodology) and the occurrence of neural tube defects in the children of US Viet Nam War veterans (219) would have been missed.

The number of studies examining biomarkers of genetic susceptibility for adverse reproductive outcomes is growing rapidly, with important implications for prenatal screening, as well as for the detection of gene–environment interactions. In addition to new challenges regarding the acquisition and ethical utilization of biological samples, there are longstanding constraints posed by the conventional approaches to data analysis that measure gene–environment interactions on a multiplicative rather than additive scale (e.g. relative risk versus risk difference) and require extremely large sample sizes (222–226).

Future directions and challenges

In addition to the development of biomarkers using easily obtained biological samples, research into the identification of biomarkers that would enable detection when fertilization occurs would enhance our understanding of fecundability and fertility as related to environmental and lifestyle factors. Identification of additional genetic susceptibility markers will open multiple avenues of research in both the genetic screening area, as well as research into gene–environment interactions in the etiology of adverse reproductive outcomes. All of the advances will result in the increasing necessity to develop safeguards for the confidentiality and ethical use of the data obtained. It is also critical that effective means of communication of study results to participants be developed, so that important information regarding their

health status is provided to them while also taking into consideration situations in which the interpretation of the data and their relevance to reproductive health may not yet be understood.

To be sure, the vast and growing array of biomarkers and measurements that can be made at the molecular level fuels hope and raises expectations for breakthrough discoveries in reproductive epidemiology, with the potential for rapid translation and significant health benefits. At the same time, exciting developments in molecular epidemiology must be balanced by advances in research design and data analysis that foster methodologic rigor, replication and sustained scientific vigilance. Recent rulings by the US federal Vaccine Injury Compensation Program, highlighted by the Hannah Poling case alleging vaccine-induced autism, raise concern for the temptation to accept biologically plausible molecular mechanisms on the perceived elegance of the argument over the weight of the empirical evidence (227).

Future molecular epidemiology studies of reproductive and developmental health will be shaped also by the increasing pressures to register clinical research (228) and to share data within a limited time frame (229). A socioeconomic exigency of modern, multidisciplinary, multicentre epidemiologic studies (e.g. genome-wide association studies (26–33)) data sharing has broad and complex ethical implications for study participants, study investigators and other stakeholders ranging from corporate interests to the scientific community and the population at large. For future studies aiming to advance the fields of reproductive and molecular epidemiology, the ethical challenges of data sharing must be weighed against the growing demand for scientific synergies and public health benefit (229).

References

1. Selevan SG, Kimmel CA, Mendola P (2000). Identifying critical windows of exposure for children's health. *Environ Health Perspect,* 108 Suppl 3;451–455.doi:10.2307/3454536 PMID:10852844

2. Lemasters GK, Perreault SD, Hales BF *et al.* (2000). Workshop to identify critical windows of exposure for children's health: reproductive health in children and adolescents work group summary. *Environ Health Perspect,* 108 Suppl 3;505–509.doi:10.2307/3454542 PMID:10852850

3. Pryor JL, Hughes C, Foster W *et al.* (2000). Critical windows of exposure for children's health: the reproductive system in animals and humans. *Environ Health Perspect,* 108 Suppl 3;491–503.doi:10.2307/3454541 PMID: 10852849

4. Olshan AF, Anderson L, Roman E *et al.* (2000). Workshop to identify critical windows of exposure for children's health: cancer work group summary. *Environ Health Perspect,* 108 Suppl 3;595–597.doi:10.2307/3454550 PMID:10852858

5. Barr M Jr, DeSesso JM, Lau CS *et al.* (2000). Workshop to identify critical windows of exposure for children's health: cardiovascular and endocrine work group summary. *Environ Health Perspect,* 108 Suppl 3;569–571. doi:10.2307/3454548 PMID:10852856

6. Adams J, Barone S Jr, LaMantia A *et al.* (2000). Workshop to identify critical windows of exposure for children's health: neurobehavioral work group summary. *Environ Health Perspect,* 108 Suppl 3;535–544.doi:10.2307/3454544 PMID:10852852

7. Dietert RR, Etzel RA, Chen D *et al.* (2000). Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary. *Environ Health Perspect,* 108 Suppl 3;483–490.doi:10.2307/3454540 PMID:10852848

8. Buck GM, Lynch CD, Stanford JB *et al.* (2004). Prospective pregnancy study designs for assessing reproductive and developmental toxicants. *Environ Health Perspect,* 112:79–86.doi:10.1289/ehp.6262 PMID:14698935

9. Lasley BL, Overstreet JW (1998). Biomarkers for assessing human female reproductive health, an interdisciplinary approach. *Environ Health Perspect,* 106 Suppl 4;955–960. PMID:9703478

10. Rockett JC, Buck GM, Lynch CD, Perreault SD (2004). The value of home-based collection of biospecimens in reproductive epidemiology. *Environ Health Perspect,* 112:94–104. doi:10.1289/ehp.6264 PMID:14698937

11. Lemasters GK, Schulte PA. Biologic markers in the epidemiology of reproduction. In: Schulte PA, Perera FP, editors. Molecular epidemiology: principles and practices; 1993. p. 385–406.

12. Wilson SH, Suk WA. Biomarkers of environmentally associated disease: technologies, concepts and perspectives. Boca Raton (FL): Lewis Publishers; 2002.

13. National Research Council. Hormonally active agents in the environment. Washington (DC): National Academy Press; 1999.

14. Perera FP, Herbstman JB (2008). Emerging technology in molecular epidemiology: what epidemiologists need to know. *Epidemiology,* 19:350–352.doi:10.1097/EDE.0b013e318162a920 PMID:18277170

15. Thompson RCA. Molecular epidemiology of infectious diseases. New York (NY): Arnold (Co-published by Oxford University Press); 2000.

16. Riley LA. Molecular epidemiology of infectious diseases: principles and practices. Washington (DC): ASM Press; 2004.

17. Foxman B (2007). Contributions of molecular epidemiology to the understanding of infectious disease transmission, pathogenesis, and evolution. *Ann Epidemiol,* 17:148–156.doi:10.1016/j.annepidem.2006.09.004 PMID:17175168

18. Wigle DT, Arbuckle TE, Turner MC *et al.* (2008). Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. *J Toxicol Environ Health B Crit Rev,* 11:373–517. PMID:18470797

19. Rockett JC, Kim SJ (2005). Biomarkers of reproductive toxicity. *Cancer Biomark,* 1:93–108. PMID:17192035

20. Ong CN, Shen HM, Chia SE (2002). Biomarkers for male reproductive health hazards: are they available? *Toxicol Lett,* 134:17–30.doi:10.1016/S0378-4274(02)00159-5 PMID:12191857

21. Burke W, Khoury MJ, Stewart A, Zimmern RL; Bellagio Group (2006). The path from genome-based research to population health: development of an international public health genomics network. *Genet Med,* 8:451–458. doi:10.1097/01.gim.0000228213.72256.8c PMID:16845279

22. Seminara D, Khoury MJ, O'Brien TR *et al.*; Human Genome Epidemiology Network; Network of Investigator Networks (2007). The emergence of networks in human genome epidemiology: challenges and opportunities. *Epidemiology*, 18:1–8.doi:10.1097/01.ede.0000249540.17855.b7 PMID:17179752

23. Field D, Sansone SA (2006). A special issue on data standards. *Omics: a journal of integrative biology*, 10(2):84–93.

24. Ho SM, Tang WY (2007). Techniques used in studies of epigenome dysregulation due to aberrant DNA methylation: an emphasis on fetal-based adult diseases. *Reprod Toxicol,* 23:267–282.doi:10.1016/j.reprotox.2007.01.004 PMID:17317097

25. Beyer A, Bandyopadhyay S, Ideker T (2007). Integrating physical and genetic maps: from genomes to interaction networks. *Nat Rev Genet,* 8:699–710.doi:10.1038/nrg2144 PMID:17703239

26. Aston KI, Carrell DT (2009). Genome-wide study of single-nucleotide polymorphisms associated with azoospermia and severe oligozoospermia. *J Androl,* 30:711–725.doi:10.2164/jandrol.109.007971 PMID:19478329

27. Beaty TH, Murray JC, Marazita ML *et al.* (2010). A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. *Nat Genet,* 42:525–529.doi:10.1038/ng.580 PMID:20436469

28. Freathy RM, Mook-Kanamori DO, Sovio U *et al.*; Genetic Investigation of ANthropometric Traits (GIANT) Consortium; Meta-Analyses of Glucose and Insulin-related traits Consortium; Wellcome Trust Case Control Consortium; Early Growth Genetics (EGG) Consortium (2010). Variants in ADCY5 and near CCNL1 are associated with fetal growth and birth weight. *Nat Genet,* 42:430–435.doi:10.1038/ng.567 PMID:20372150

29. Ingersoll RG, Hetmanski J, Park JW *et al.* (2010). Association between genes on chromosome 4p16 and non-syndromic oral clefts in four populations. *Eur J Hum Genet,* 18:726–732.doi:10.1038/ejhg.2009.228 PMID:20087401

30. Liu YZ, Guo YF, Wang L *et al.* (2009). Genome-wide association analyses identify SPOCK as a key novel gene underlying age at menarche. *PLoS Genet,* 5:e1000420. doi:10.1371/journal.pgen.1000420 PMID:19282985

31. Loos RJ, Lindgren CM, Li S *et al.*; Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial; KORA; Nurses' Health Study; Diabetes Genetics Initiative; SardiNIA Study; Wellcome Trust Case Control Consortium; FUSION (2008). Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nat Genet,* 40:768–775. doi:10.1038/ng.140 PMID:18454148

32. Mangold E, Ludwig KU, Birnbaum S *et al.* (2010). Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. *Nat Genet,* 42:24–26.doi:10.1038/ng.506 PMID:20023658

33. Voorhuis M, Onland-Moret NC, van der Schouw YT *et al.* (2010). Human studies on genetics of the age at natural menopause: a systematic review. *Hum Reprod Update,* 16:364–377.doi:10.1093/humupd/dmp055 PMID:20071357

34. Herman-Giddens ME (2006). Recent data on pubertal milestones in United States children: the secular trend toward earlier development. *Int J Androl,* 29:241–246, discussion 286–290.doi:10.1111/j.1365-2605.2005.00575.x PMID:16466545

35. Euling SY, Herman-Giddens ME, Lee PA *et al.* (2008). Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics,* 121 Suppl 3;S172–S191.doi:10.1542/peds.2007-1813D PMID:18245511

36. Tanner JM. Growth at adolescence. Oxford: Blackwell Scientific; 1962.

37. Duke PM, Litt IF, Gross RT (1980). Adolescents' self-assessment of sexual maturation. *Pediatrics*, 66:918–920. PMID:7454482

38. Neinstein LS (1982). Adolescent self-assessment of sexual maturation: reassessment and evaluation in a mixed ethnic urban population. *Clin Pediatr (Phila),* 21:482–484.doi:10.1177/000992288202100806 PMID:7083719

39. Wu T, Mendola P, Buck GM (2002). Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: the Third National Health and Nutrition Examination Survey, 1988–1994. *Pediatrics*, 110:752–757.doi:10.1542/peds.110.4.752 PMID:12359790

40. Rockett JC, Lynch CD, Buck GM (2004). Biomarkers for assessing reproductive development and health: Part 1–Pubertal development. *Environ Health Perspect,* 112:105–112.doi:10.1289/ehp.6265 PMID:14698938

41. Blanck HM, Marcus M, Tolbert PE *et al.* (2000). Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl. *Epidemiology*, 11:641–647.doi:10.1097/00001648-200011000-00005 PMID:11055623

42. Mantzoros CS, Flier JS, Rogol AD (1997). A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. *J Clin Endocrinol Metab,* 82:1066–1070.doi:10.1210/jc.82.4.1066 PMID:9100574

43. Roemmich JN, Rogol AD (1999). Role of leptin during childhood growth and development. [viii.]. *Endocrinol Metab Clin North Am,* 28:749–764, viii.doi:10.1016/S0889-8529(05)70100-6 PMID:10609118

44. Lee MM, Donahoe PK, Hasegawa T *et al.* (1996). Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. *J Clin Endocrinol Metab*, 81:571–576.doi:10.1210/jc.81.2.571 PMID:8636269

45. Kiess W, Reich A, Meyer K *et al.* (1999). A role for leptin in sexual maturation and puberty? *Horm Res,* 51 Suppl 3;55–63. doi:10.1159/000053163 PMID:10592445

46. Zaman N, Hall CM, Gill MS *et al.* (2003). Leptin measurement in urine in children and its relationship to other growth peptides in serum and urine. *Clin Endocrinol (Oxf),* 58:78–85. doi:10.1046/j.1365-2265.2003.01677.x PMID:12519416

47. Wu FC, Brown DC, Butler GE *et al.* (1993). Early morning plasma testosterone is an accurate predictor of imminent pubertal development in prepubertal boys. *J Clin Endocrinol Metab,* 76:26–31.doi:10.1210/jc.76.1.26 PMID:8421096

48. Demir A, Dunkel L, Stenman UH, Voutilainen R (1995). Age-related course of urinary gonadotropins in children. *J Clin Endocrinol Metab*, 80:1457–1460.doi:10.1210/jc.80.4.1457 PMID:7714124

49. Maqsood AR, Trueman JA, Whatmore AJ *et al.* (2007). The relationship between nocturnal urinary leptin and gonadotrophins as children progress towards puberty. *Horm Res,* 68:225–230.doi:10.1159/000101335 PMID:17389812

50. Rajpert-De Meyts E, Jørgensen N, Graem N *et al.* (1999). Expression of anti-Müllerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. *J Clin Endocrinol Metab,* 84:3836–3844.doi:10.1210/jc.84.10.3836 PMID:10523039

51. Josso N, Cate RL, Picard JY *et al.* (1993). Anti-müllerian hormone: the Jost factor. *Recent Prog Horm Res,* 48:1–59. PMID:8441845

52. Lee MM, Donahoe PK (1993). Mullerian inhibiting substance: a gonadal hormone with multiple functions. *Endocr Rev,* 14:152–164. PMID:8325249

53. Hudson PL, Dougas I, Donahoe PK *et al.* (1990). An immunoassay to detect human müllerian inhibiting substance in males and females during normal development. *J Clin Endocrinol Metab,* 70:16–22.doi:10.1210/jcem-70-1-16 PMID:2294129

54. Chada M, Průsa R, Bronský J *et al.* (2003). Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone during childhood and puberty in males: changes in serum concentrations in relation to age and stage of puberty. *Physiol Res,* 52:45–51. PMID:12625806

55. Radicioni AF, Anzuini A, De Marco E *et al.* (2005). Changes in serum inhibin B during normal male puberty. *Eur J Endocrinol,* 152:403–409.doi:10.1530/eje.1.01855 PMID:15757857

56. Crofton PM, Evans AE, Groome NP *et al.* (2002). Dimeric inhibins in girls from birth to adulthood: relationship with age, pubertal stage, FSH and oestradiol. *Clin Endocrinol (Oxf),* 56:223–230.doi:10.1046/j.0300-0664.2001.01449.x PMID:11874414

57. Foster CM, Olton PR, Padmanabhan V (2005). Diurnal changes in FSH-regulatory peptides and their relationship to gonadotrophins in pubertal girls. *Hum Reprod,* 20:543–548.doi:10.1093/humrep/deh607 PMID:15550493

58. Rogan WJ, Ragan NB (2007). Some evidence of effects of environmental chemicals on the endocrine system in children. *Int J Hyg Environ Health,* 210:659–667.doi:10.1016/j.ijheh.2007.07.005 PMID:17870664

59. Den Hond E, Schoeters G (2006). Endocrine disrupters and human puberty. *Int J Androl,* 29:264–271, discussion 286–290.doi:10.1111/j.1365-2605.2005.00561.x PMID:16466548

60. Schoeters G, Den Hond E, Dhooge W *et al.* (2008). Endocrine disruptors and abnormalities of pubertal development. *Basic Clin Pharmacol Toxicol,* 102:168–175.doi:10.1111/j.1742-7843.2007.00180.x PMID:18226071

61. Wolff MS, Britton JA, Boguski L *et al.* (2008). Environmental exposures and puberty in inner-city girls. *Environ Res,* 107:393–400. doi:10.1016/j.envres.2008.03.006 PMID:18479682

62. Buck Louis GM, Gray LE Jr, Marcus M *et al.* (2008). Environmental factors and puberty timing: expert panel research needs. *Pediatrics,* 121 Suppl 3;S192–S207. doi:10.1542/peds.1813E PMID:18245512

63. Kavlock RJ, Daston GP, DeRosa C *et al.* (1996). Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. *Environ Health Perspect,* 104 Suppl 4;715–740. PMID:8880000

64. Graziottin A (2000). Libido: the biologic scenario. *Maturitas*, 34 Suppl 1;S9–S16. doi:10.1016/S0378-5122(99)00072-9 PMID:10759059

65. McHorney CA, Rust J, Golombok S *et al.* (2004). Profile of Female Sexual Function: a patient-based, international, psychometric instrument for the assessment of hypoactive sexual desire in oophorectomized women. *Menopause*, 11:474–483.doi:10.1097/01.GME.0000109316.11228.77 PMID:15243286

66. Bancroft J, Cawood EH (1996). Androgens and the menopause; a study of 40–60-year-old women. *Clin Endocrinol (Oxf)*, 45:577–587.doi:10.1046/j.1365-2265.1996.00846.x PMID:8977755

67. Avis NE, Stellato R, Crawford S *et al.* (2000). Is there an association between menopause status and sexual functioning? *Menopause*, 7:297–309.doi:10.1097/00042192-200007050-00004 PMID:10993029

68. Dennerstein L, Dudley E, Burger H (2001). Are changes in sexual functioning during midlife due to aging or menopause? *Fertil Steril,* 76:456–460.doi:10.1016/S0015-0282(01)01978-1 PMID:11532464

69. Basson R, Berman J, Burnett A *et al.* (2000). Report of the international consensus development conference on female sexual dysfunction: definitions and classifications. *J Urol,* 163:888–893.doi:10.1016/S0022-5347(05)67828-7 PMID:10688001

70. Bancroft J (2002). The medicalization of female sexual dysfunction: the need for caution. *Arch Sex Behav,* 31:451–455. doi:10.1023/A:1019800426980 PMID:12238614

71. Leiblum SR, Koochaki PE, Rodenberg CA *et al.* (2006). Hypoactive sexual desire disorder in postmenopausal women: US results from the Women's International Study of Health and Sexuality (WISHeS). *Menopause*, 13:46–56.doi:10.1097/01.gme.0000172596.76272.06 PMID:16607098

72. Laumann EO, Paik A, Rosen RC (1999). Sexual dysfunction in the United States: prevalence and predictors. *JAMA*, 281:537–544.doi:10.1001/jama.281.6.537 PMID:10022110

73. Turna B, Apaydin E, Semerci B *et al.* (2005). Women with low libido: correlation of decreased androgen levels with female sexual function index. *Int J Impot Res*, 17:148–153. doi:10.1038/sj.ijir.3901294 PMID:15592425

74. Nyunt A, Stephen G, Gibbin J *et al.* (2005). Androgen status in healthy premenopausal women with loss of libido. *J Sex Marital Ther,* 31:73–80.doi:10.1080/00926230590475314 PMID:15841707

75. Granger DA, Shirtcliff EA, Booth A *et al.* (2004). The "trouble" with salivary testosterone. *Psychoneuroendocrinology,* 29:1229–1240. doi:10.1016/j.psyneuen.2004.02.005 PMID:15288702

76. Whembolua GL, Granger DA, Singer S *et al.* (2006). Bacteria in the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Horm Behav*, 49:478–483.doi:10.1016/j.yhbeh.2005.10.005 PMID:16309679

77. Baird DD (1999). Characteristics of fertile menstrual cycles. *Scand J Work Environ Health,* 25 Suppl 1;20–22, discussion 76–78. PMID:10235401

78. van Zonneveld P, Scheffer GJ, Broekmans FJ *et al.* (2003). Do cycle disturbances explain the age-related decline of female fertility? Cycle characteristics of women aged over 40 years compared with a reference population of young women. *Hum Reprod,* 18:495–501. doi:10.1093/humrep/deg138 PMID:12615813

79. Li H, Nakajima ST, Chen J *et al.* (2001). Differences in hormonal characteristics of conceptive versus nonconceptive menstrual cycles. *Fertil Steril,* 75:549–553.doi:10.1016/S0015-0282(00)01765-9 PMID:11239540

80. Small CM, Manatunga AK, Klein M *et al.* (2006). Menstrual cycle characteristics: associations with fertility and spontaneous abortion. *Epidemiology*, 17:52–60.doi:10.1097/01.ede.0000190540.95748.e6 PMID:16357595

81. Waller K, Swan SH, Windham GC *et al.* (1998). Use of urine biomarkers to evaluate menstrual function in healthy premenopausal women. *Am J Epidemiol,* 147:1071–1080. PMID:9620051

82. Windham GC, Elkin E, Fenster L *et al.* (2002). Ovarian hormones in premenopausal women: variation by demographic, reproductive and menstrual cycle characteristics. *Epidemiology*, 13:675–684. doi:10.1097/00001648-200211000-00012 PMID:12410009

83. Liu Y, Gold EB, Lasley BL, Johnson WO (2004). Factors affecting menstrual cycle characteristics. *Am J Epidemiol,* 160:131–140.doi:10.1093/aje/kwh188 PMID:15234934

84. Harlow SD, Campbell B (1996). Ethnic differences in the duration and amount of menstrual bleeding during the postmenarcheal period. *Am J Epidemiol,* 144:980–988. PMID:8916509

85. Harlow SD, Campbell B, Lin X, Raz J (1997). Ethnic differences in the length of the menstrual cycle during the postmenarcheal period. *Am J Epidemiol,* 146:572–580. PMID:9326435

86. Rowland AS, Baird DD, Long S *et al.* (2002). Influence of medical conditions and lifestyle factors on the menstrual cycle. *Epidemiology*, 13:668–674.doi:10.1097/00001648-200211000-00011 PMID:12410008

87. Chatterton RT Jr, Mateo ET, Hou N *et al.* (2005). Characteristics of salivary profiles of oestradiol and progesterone in premenopausal women. *J Endocrinol,* 186:77–84.doi:10.1677/joe.1.06025 PMID:16002538

88. Zinaman MJ (2006). Using cervical mucus and other easily observed biomarkers to identify ovulation in prospective pregnancy trials. *Paediatr Perinat Epidemiol,* 20 Suppl 1;26–29.doi:10.1111/j.1365-3016.2006.00767.x PMID:17061970

89. Gann PH, Giovanazzi S, Van Horn L *et al.* (2001). Saliva as a medium for investigating intra- and interindividual differences in sex hormone levels in premenopausal women. *Cancer Epidemiol Biomarkers Prev,* 10:59–64. PMID:11205490

90. Lu Y, Bentley GR, Gann PH *et al.* (1999). Salivary estradiol and progesterone levels in conception and nonconception cycles in women: evaluation of a new assay for salivary estradiol. *Fertil Steril,* 71:863–868. doi:10.1016/S0015-0282(99)00093-X PMID: 10231047

91. Lipson SF, Ellison PT (1996). Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. *Hum Reprod,* 11:2090–2096. PMID:8943508

92. Hardy K, Wright C, Rice S *et al.* (2002). Future developments in assisted reproduction in humans. *Reproduction*, 123:171–183.doi:10.1530/rep.0.1230171 PMID:11866685

93. Goldberg JM, Falcone T, Attaran M (2007). In vitro fertilization update. *Cleve Clin J Med,* 74:329–338.doi:10.3949/ccjm.74.5.329 PMID:17506238

94. Gleicher N, Weghofer A, Barad D (2008). Preimplantation genetic screening: "established" and ready for prime time? *Fertil Steril,* 89:780–788.doi:10.1016/j.fertnstert.2008.01.072 PMID:18353323

95. Canfield RE, O'Connor JF, Birken S *et al.* (1987). Development of an assay for a biomarker of pregnancy and early fetal loss. *Environ Health Perspect,* 74:57–66. doi:10.1289/ehp.877457 PMID:3319556

96. Wilcox AJ, Weinberg CR, O'Connor JF *et al.* (1988). Incidence of early loss of pregnancy. *N Engl J Med,* 319:189–194.doi:10.1056/NEJM198807283190401 PMID:3393170

97. Sweeney AM, Meyer MR, Aarons JH *et al.* (1988). Evaluation of methods for the prospective identification of early fetal losses in environmental epidemiology studies. *Am J Epidemiol,* 127:843–850. PMID:3354549

98. Lasley BL, Lohstroh P, Kuo A *et al.* (1995). Laboratory methods for evaluating early pregnancy loss in an industry-based population. *Am J Ind Med,* 28:771–781.doi:10.1002/ajim.4700280611 PMID:8588563

99. Stewart DR, Overstreet JW, Nakajima ST, Lasley BL (1993). Enhanced ovarian steroid secretion before implantation in early human pregnancy. *J Clin Endocrinol Metab,* 76:1470–1476.doi:10.1210/jc.76.6.1470 PMID:8501152

100. Qiu Q, Overstreet JW, Todd H *et al.* (1997). Total urinary follicle stimulating hormone as a biomarker for detection of early pregnancy and periimplantation spontaneous abortion. *Environ Health Perspect,* 105:862–866.doi:10.1289/ehp.97105862 PMID:9347902

101. Lohstroh PN, Overstreet JW, Stewart DR *et al.* (2005). Secretion and excretion of human chorionic gonadotropin during early pregnancy. *Fertil Steril,* 83:1000–1011.doi:10.1016/j.fertnstert.2004.10.038 PMID:15 820813

102. Vitzthum VJ, Spielvogel H, Thornburg J, West B (2006). A prospective study of early pregnancy loss in humans. *Fertil Steril,* 86:373–379.doi:10.1016/j.fertnstert.2006.01.021 PMID:16806213

103. Pyper C, Bromhall L, Dummett S *et al.* (2006). The Oxford Conception Study design and recruitment experience. *Paediatr Perinat Epidemiol,* 20 Suppl 1;51–59.doi:10.1111/j.1365-3016.2006.00771.x PMID:17061974

104. Lynch CD, Jackson LW, Buck Louis GM (2006). Estimation of the day-specific probabilities of conception: current state of the knowledge and the relevance for epidemiological research. *Paediatr Perinat Epidemiol,* 20 Suppl 1;3–12.doi:10.1111/j.1365-3016.2006.00765.x PMID:17061968

105. Morton H, Rolfe B, Clunie GJ (1977). An early pregnancy factor detected in human serum by the rosette inhibition test. *Lancet*, 1:394–397.doi:10.1016/S0140-6736(77)92605-8 PMID:65512

106. Fan XG, Zheng ZQ (1997). A study of early pregnancy factor activity in preimplantation. *Am J Reprod Immunol,* 37:359–364. PMID:9196793

107. Cavanagh AC, Morton H, Rolfe BE, Gidley-Baird AA (1982). Ovum factor: a first signal of pregnancy? *Am J Reprod Immunol,* 2:97–101. PMID:7102890

108. Cheng SJ, Zheng ZQ (2004). Early pregnancy factor in cervical mucus of pregnant women. *Am J Reprod Immunol*, 51:102–105. doi:10.1046/j.8755-8920.2003.00136.x PMID:14748834

109. Olsen J, Basso O. Reproductive epidemiology. In: Ahrens W, Pigeot I, editors. Handbook of epidemiology. Berlin: Springer; 2005. p. 1043–109.

110. Baumann MU, Bersinger NA, Surbek DV (2007). Serum markers for predicting pre-eclampsia. *Mol Aspects Med,* 28:227–244. doi:10.1016/j.mam.2007.04.002 PMID:17532461

111. Papageorghiou AT (2008). Predicting and preventing pre-eclampsia-where to next? *Ultrasound Obstet Gynecol,* 31:367–370. doi:10.1002/uog.5320 PMID:18383482

112. Murphy DJ, Fowlie PW, McGuire W (2004). Obstetric issues in preterm birth. *BMJ*, 329:783–786.doi:10.1136/bmj.329.7469.783 PMID:15459053

113. Breathnach FM, Malone FD, Lambert-Messerlian G *et al.*; First and Second Trimester Evaluation of Risk (FASTER) Research Consortium (2007). First- and second-trimester screening: detection of aneuploidies other than Down syndrome. *Obstet Gynecol,* 110:651–657.doi:10.1097/01.AOG.0000278570.76392.a6 PMID:17766613

114. Spencer K (2007). Aneuploidy screening in the first trimester. *Am J Med Genet C Semin Med Genet*, 145C:18–32. PMID:17290444

115. Wald NJ, Rudnicka AR, Bestwick JP (2006). Sequential and contingent prenatal screening for Down syndrome. *Prenat Diagn,* 26:769–777.doi:10.1002/pd.1498 PMID:16821246

116. Rosen T, D'Alton ME, Platt LD, Wapner R; Nuchal Translucency Oversight Committee, Maternal Fetal Medicine Foundation (2007). First-trimester ultrasound assessment of the nasal bone to screen for aneuploidy. *Obstet Gynecol,* 110:399–404.doi:10.1097/01.AOG.0000275281.19344.66 PMID:17666617

117. Scott A (2007). Nuchal translucency measurement in first trimester Down syndrome screening. *Issues Emerg Health Technol,* (100):1–6. PMID:17595751

118. Sekizawa A, Purwosunu Y, Matsuoka R *et al.* (2007). Recent advances in non-invasive prenatal DNA diagnosis through analysis of maternal blood. *J Obstet Gynaecol Res*, 33:747–764.doi:10.1111/j.1447-0756.2007.00652.x PMID:18001438

119. Torday JS, Rehan VK (2003). Testing for fetal lung maturation: a biochemical "window" to the developing fetus. *Clin Lab Med*, 23:361–383.doi:10.1016/S0272-2712(03)00030-1 PMID:12848449

120. Rubin LP, Hansen K (2003). Testing for hematologic disorders and complications. *Clin Lab Med,* 23:317–343.doi:10.1016/S0272-2712(03)00031-3 PMID:12848447

121. Lightfoot T, Bunch K, Ansell P, Murphy M (2005). Ovulation induction, assisted conception and childhood cancer. *Eur J Cancer,* 41:715–724, discussion 725–726. doi:10.1016/j.ejca.2004.07.032 PMID:15763647

122. Cheung AP (2006). Assisted reproductive technology: both sides now. *J Reprod Med,* 51:283–292. PMID:16739266

123. Knoester M, Helmerhorst FM, Vandenbroucke JP *et al.*; Leiden Artificial Reproductive Techniques Follow-up Project (L-art-FUP) (2008). Perinatal outcome, health, growth, and medical care utilization of 5- to 8-year-old intracytoplasmic sperm injection singletons. *Fertil Steril,* 89:1133–1146.doi:10.1016/j.fertnstert.2007.04.049 PMID:18177652

124. Parker SP, Khan HI, Cubitt WD (1999). Detection of antibodies to hepatitis C virus in dried blood spot samples from mothers and their offspring in Lahore, Pakistan. *J Clin Microbiol,* 37:2061–2063. PMID:10325381

125. Wiemels JL, Cazzaniga G, Daniotti M *et al.* (1999). Prenatal origin of acute lymphoblastic leukaemia in children. *Lancet*, 354:1499–1503.doi:10.1016/S0140-6736(99)09403-9 PMID:10551495

126. Crawford DC, Caggana M, Harris KB *et al.* (2002). Characterization of beta-globin haplotypes using blood spots from a population-based cohort of newborns with homozygous HbS. *Genet Med,* 4:328–335. doi:10.1097/00125817-200209000-00003 PMID:12394345

127. Klotz J, Bryant P, Wilcox HB *et al.* (2006). Population-based retrieval of newborn dried blood spots for researching paediatric cancer susceptibility genes. *Paediatr Perinat Epidemiol,* 20:449–452.doi:10.1111/j.1365-3016.2006.00749.x PMID:16911024

128. Spector LG, Hecht SS, Ognjanovic S *et al.* (2007). Detection of cotinine in newborn dried blood spots. *Cancer Epidemiol Biomarkers Prev,* 16:1902–1905.doi:10.1158/1055-9965.EPI-07-0230 PMID:17855712

129. Patton JC, Akkers E, Coovadia AH *et al.* (2007). Evaluation of dried whole blood spots obtained by heel or finger stick as an alternative to venous blood for diagnosis of human immunodeficiency virus type 1 infection in vertically exposed infants in the routine diagnostic laboratory. *Clin Vaccine Immunol,* 14:201–203.doi:10.1128/CVI.00223-06 PMID:17167036

130. Olshan AF (2007). Meeting report: the use of newborn blood spots in environmental research: opportunities and challenges. *Environ Health Perspect,* 115:1767–1779.doi:10.1289/ehp.10511 PMID:18087597

131. Mei JV, Alexander JR, Adam BW, Hannon WH (2001). Use of filter paper for the collection and analysis of human whole blood specimens. *J Nutr*, 131:1631S–1636S. PMID:11340130

132. Mandl KD, Feit S, Larson C, Kohane IS (2002). Newborn screening program practices in the United States: notification, research, and consent. *Pediatrics*, 109:269–273.doi:10.1542/peds.109.2.269 PMID:11826206

133. Olney RS, Moore CA, Ojodu JA *et al.* (2006). Storage and use of residual dried blood spots from state newborn screening programs. *J Pediatr,* 148:618–622.doi:10.1016/j.jpeds.2005.12.053 PMID:16737872

134. Ge RS, Chen GR, Tanrikut C, Hardy MP (2007). Phthalate ester toxicity in Leydig cells: developmental timing and dosage considerations. *Reprod Toxicol,* 23:366–373. doi:10.1016/j.reprotox.2006.12.006 PMID:17258888

135. Martin OV, Shialis T, Lester JN *et al.* (2008). Testicular dysgenesis syndrome and the estrogen hypothesis: a quantitative meta-analysis. *Environ Health Perspect,* 116:149–157.doi:10.1289/ehp.10545 PMID:18288311

136. Schull WJ (2003). The children of atomic bomb survivors: a synopsis. *J Radiol Prot,* 23:369–384.doi:10.1088/0952-4746/23/4/R302 PMID:14750686

137. Signorello LB, Cohen SS, Bosetti C *et al.* (2006). Female survivors of childhood cancer: preterm birth and low birth weight among their children. *J Natl Cancer Inst,* 98:1453–1461. doi:10.1093/jnci/djj394 PMID:17047194

138. Nagarajan R, Robison LL (2005). Pregnancy outcomes in survivors of childhood cancer. *J Natl Cancer Inst Monogr,* 2005:72–76.doi:10.1093/jncimonographs/lgi020 PMID:15784829

139. Botto LD, Yang Q (2000). 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. *Am J Epidemiol,* 151:862–877. PMID:10791559

140. Finnell RH, Shaw GM, Lammer EJ *et al.* (2004). Gene-nutrient interactions: importance of folates and retinoids during early embryogenesis. *Toxicol Appl Pharmacol,* 198:75–85.doi:10.1016/j.taap.2003.09.031 PMID:15236946

141. Blom HJ, Shaw GM, den Heijer M, Finnell RH (2006). Neural tube defects and folate: case far from closed. *Nat Rev Neurosci,* 7:724–731.doi:10.1038/nrn1986 PMID:16924 261

142. Zeiger JS, Beaty TH, Liang KY (2005). Oral clefts, maternal smoking, and TGFA: a meta-analysis of gene-environment interaction. *Cleft Palate Craniofac J,* 42:58–63.doi:10.1597/02-128.1 PMID:15643916

143. Krapels IP, Raijmakers-Eichhorn J, Peters WH *et al.*; Eurocran Gene-Environment Interaction Group (2008). The I,105V polymorphism in glutathione S-transferase P1, parental smoking and the risk for nonsyndromic cleft lip with or without cleft palate. *Eur J Hum Genet,* 16:358–366. doi:10.1038/sj.ejhg.5201973 PMID:18159215

144. Lie RT, Wilcox AJ, Taylor J *et al.* (2008). Maternal smoking and oral clefts: the role of detoxification pathway genes. *Epidemiology*, 19:606–615.doi:10.1097/EDE.0b013e3181690731 PMID:18449058

145. Torfs CP, Christianson RE, Iovannisci DM *et al.* (2006). Selected gene polymorphisms and their interaction with maternal smoking, as risk factors for gastroschisis. *Birth Defects Res A Clin Mol Teratol,* 76:723–730. doi:10.1002/bdra.20310 PMID:17051589

146. Kishi R, Sata F, Yoshioka E *et al.* (2008). Exploiting gene-environment interaction to detect adverse health effects of environmental chemicals on the next generation. *Basic Clin Pharmacol Toxicol,* 102:191–203.doi:10.1111/j.1742-7843.2007.00201.x PMID:18226074

147. Etheredge AJ, Christensen K, Del Junco D *et al.* (2005). Evaluation of two methods for assessing gene-environment interactions using data from the Danish case-control study of facial clefts. *Birth Defects Res A Clin Mol Teratol,* 73:541–546.doi:10.1002/bdra.20167 PMID:15965987

148. Smith GD (2008). Assessing intrauterine influences on offspring health outcomes: can epidemiological studies yield robust findings? *Basic Clin Pharmacol Toxicol,* 102:245–256.doi:10.1111/j.1742-7843.2007.00191.x PMID:18226080

149. Tsai HJ, Liu X, Mestan K *et al.* (2008). Maternal cigarette smoking, metabolic gene polymorphisms, and preterm delivery: new insights on GxE interactions and pathogenic pathways. *Hum Genet,* 123:359–369.doi:10.1007/s00439-008-0485-9PMID:18320 229

150. Wang X, Zuckerman B, Pearson C *et al.* (2002). Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *JAMA*, 287:195–202.doi:10.1001/jama.287.2.195 PMID:11779261

151. Williams TA, Mars AE, Buyske SG *et al.* (2007). Risk of autistic disorder in affected offspring of mothers with a glutathione S-transferase P1 haplotype. *Arch Pediatr Adolesc Med,* 161:356–361.doi:10.1001/archpedi.161.4.356 PMID:17404132

152. Todd RD, Neuman RJ (2007). Gene-environment interactions in the development of combined type ADHD: evidence for a synapse-based model. *Am J Med Genet B Neuropsychiatr Genet,* 144B:971–975. PMID:17955458

153. Infante-Rivard C, Weichenthal S (2007). Pesticides and childhood cancer: an update of Zahm and Ward's 1998 review. *J Toxicol Environ Health B Crit Rev,* 10:81–99. PMID:18074305

154. Newbold RR, Hanson RB, Jefferson WN *et al.* (1998). Increased tumors but uncompromised fertility in the female descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis*, 19:1655–1663.doi:10.1093/carcin/19.9.1655 PMID:9771938

155. Newbold RR, Hanson RB, Jefferson WN *et al.* (2000). Proliferative lesions and reproductive tract tumors in male descendants of mice exposed developmentally to diethylstilbestrol. *Carcinogenesis*, 21:1355–1363.doi:10.1093/carcin/21.7.1355 PMID:10874014

156. Tomatis L (1994). Transgeneration carcinogenesis: a review of the experimental and epidemiological evidence. *Jpn J Cancer Res*, 85:443–454. PMID:8014100

157. Klip H, Verloop J, van Gool JD *et al.*; OMEGA Project Group (2002). Hypospadias in sons of women exposed to diethylstilbestrol in utero: a cohort study. *Lancet*, 359:1102–1107.doi:10.1016/S0140-6736(02)08152-7 PMID:11943257

158. Palmer JR, Wise LA, Robboy SJ *et al.* (2005). Hypospadias in sons of women exposed to diethylstilbestrol in utero. *Epidemiology*, 16:583–586.doi:10.1097/01.ede.0000164789.59728.6d PMID:15951681

159. Pons JC, Papiernik E, Billon A *et al.* (2005). Hypospadias in sons of women exposed to diethylstilbestrol in utero. *Prenat Diagn*, 25:418–419.doi:10.1002/pd.1136 PMID:15906411

160. Brouwers MM, Feitz WF, Roelofs LA *et al.* (2006). Hypospadias: a transgenerational effect of diethylstilbestrol? *Hum Reprod,* 21:666–669.doi:10.1093/humrep/dei398 PMID:16293648

161. Stoll C, Alembik Y, Dott B (2003). Limb reduction defects in the first generation and deafness in the second generation of intrauterine exposed fetuses to diethylstilbestrol. *Ann Genet,* 46:459–465. PMID:14659782

162. Blatt J, Van Le L, Weiner T, Sailer S (2003). Ovarian carcinoma in an adolescent with transgenerational exposure to diethylstilbestrol. *J Pediatr Hematol Oncol,* 25:635–636.doi:10.1097/00043426-200308000-00009 PMID:12902917

163. Li S, Hansman R, Newbold R *et al.* (2003). Neonatal diethylstilbestrol exposure induces persistent elevation of c-fos expression and hypomethylation in its exon-4 in mouse uterus. *Mol Carcinog,* 38:78–84.doi:10.1002/mc.10147 PMID:14502647

164. Waterland RA, Michels KB (2007). Epigenetic epidemiology of the developmental origins hypothesis. *Annu Rev Nutr,* 27:363–388.doi:10.1146/annurev.nutr.27.061406.093705 PMID:17465856

165. Nijland MJ, Ford SP, Nathanielsz PW (2008). Prenatal origins of adult disease. *Curr Opin Obstet Gynecol,* 20:132–138. doi:10.1097/GCO.0b013e3282f76753 PMID:18388812

166. Barker DJ (2004). The developmental origins of adult disease. *J Am Coll Nutr,* 23 Suppl;588S–595S. PMID:15640511

167. Gillman MW, Barker D, Bier D *et al.* (2007). Meeting report on the 3rd International Congress on Developmental Origins of Health and Disease (DOHaD). *Pediatr Res,* 61:625–629. PMID:17413866

168. Barker DJ (2007). The origins of the developmental origins theory. *J Intern Med,* 261:412–417.doi:10.1111/j.1365-2796.2007.01809.x PMID:17444880

169. Trosko JE, Chang CC, Upham B. Modulation of GAP junctional communication by "epigenetic" toxicants: A shared mechanism in teratogenesis, carcinogenesis, atherogenesis, immunomudulation, reproductive and neurotxicities. In: Wilson SH, Suk WA, editors. Biomarkers of environmentally associated disease: technologies, concepts and perspectives. Boca Raton (FL): Lewis Publishers; 2002. p. 445–54.

170. Dolinoy DC, Huang D, Jirtle RL (2007). Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. *Proc Natl Acad Sci USA,* 104:13056–13061.doi:10.1073/pnas.0703739104 PMID:17670942

171. Chang HS, Anway MD, Rekow SS, Skinner MK (2006). Transgenerational epigenetic imprinting of the male germline by endocrine disruptor exposure during gonadal sex determination. *Endocrinology*, 147:5524–5541.doi:10.1210/en.2006-0987 PMID:16973722

172. Ness RB, Catov J (2007). Invited commentary: Timing and types of cardiovascular risk factors in relation to offspring birth weight. *Am J Epidemiol,* 166:1365–1367.doi:10.1093/aje/kwm314 PMID:17977895

173. Golden AL. Biomarkers of male reproductive health. In: Wilson SH, Suk WA, editors. Biomarkers of environmentally associated disease: technologies, concepts, and perspectives. Boca Raton (FL): Lewis Publishers; 2002. p. 387–410.

174. Kandeel FR, Koussa VK, Swerdloff RS (2001). Male sexual function and its disorders: physiology, pathophysiology, clinical investigation, and treatment. *Endocr Rev,* 22:342–388.doi:10.1210/er.22.3.342 PMID:11399748

175. Burnett AL (2008). Environmental erectile dysfunction: can the environment really be hazardous to your erectile health? *J Androl,* 29:229–236.doi:10.2164/jandrol.107.004200 PMID:18187396

176. Schrader SM. Male reproductive toxicity. In: Massaro EJ, editor. Handbook of human toxicology. Boca Raton (FL): CRC Press; 1997. p. 962–80.

177. Webster RA. Reproductive function and pregnancy. In: McPherson RA, Pincus MR, editors. Henry's clinical diagnosis and management by laboratory methods. 21st ed. Philadelphia (PA): Saunders Elsevier; 2007. p. 364–78.

178. Mabeck LM, Jensen MS, Toft G *et al.* (2005). Fecundability according to male serum inhibin B–a prospective study among first pregnancy planners. *Hum Reprod,* 20:2909–2915.doi:10.1093/humrep/dei141 PMID:16024538

179. Ellingsen DG, Chashchin V, Haug E *et al.* (2007). An epidemiological study of reproductive function biomarkers in male welders. *Biomarkers*, 12:497–509.doi:10.1080/13547500701366496 PMID:17701748

180. Mocarelli P, Gerthoux PM, Patterson DG Jr *et al.* (2008). Dioxin exposure, from infancy through puberty, produces endocrine disruption and affects human semen quality. *Environ Health Perspect,* 116:70–77.doi:10.1289/ehp.10399 PMID:18197302

181. Dhooge W, van Larebeke N, Koppen G *et al.*; Flemish Environment and Health Study Group (2006). Serum dioxin-like activity is associated with reproductive parameters in young men from the general Flemish population. *Environ Health Perspect,* 114:1670–1676. PMID:17107851

182. Telisman S, Colak B, Pizent A *et al.* (2007). Reproductive toxicity of low-level lead exposure in men. *Environ Res,* 105:256–266. doi:10.1016/j.envres.2007.05.011 PMID:17632096

183. Sripada S, Fonseca S, Lee A *et al.* (2007). Trends in semen parameters in the northeast of Scotland. *J Androl,* 28:313– 319.doi:10.2164/jandrol.106.000729 PMID:170797 43

184. Brazil C, Swan SH, Tollner CR *et al.*; Study for Future Families Research Group (2004). Quality control of laboratory methods for semen evaluation in a multicenter research study. *J Androl,* 25:645–656. PMID:15223854

185. Ness RB, Grainger DA (2008). Male reproductive proteins and reproductive outcomes. *Am J Obstet Gynecol*, 198:620–624, e1–e4.doi:10.1016/j.ajog.2007.09.017 PMID:18191798

186. Tremellen K (2008). Oxidative stress and male infertility–a clinical perspective. *Hum Reprod Update,* 14:243–258.doi:10.1093/humupd/dmn004 PMID:18281241

187. Evenson DP, Wixon R (2006). Clinical aspects of sperm DNA fragmentation detection and male infertility. *Theriogenology*, 65:979–991.doi:10.1016/j.theriogenology.2005.09.011 PMID:16242181

188. Roeleveld N, Bretveld R (2008). The impact of pesticides on male fertility. *Curr Opin Obstet Gynecol,* 20:229–233.doi:10.1097/GCO.0b013e3282fcc334 PMID:18460936

189. Phillips KP, Tanphaichitr N (2008). Human exposure to endocrine disrupters and semen quality. *J Toxicol Environ Health B Crit Rev,* 11:188–220. PMID:18368553

190. Perry MJ (2008). Effects of environmental and occupational pesticide exposure on human sperm: a systematic review. *Hum Reprod Update,* 14:233–242.doi:10.1093/humupd/dmm039 PMID:18281240

191. Lemasters GK, Olsen DM, Yiin JH *et al.* (1999). Male reproductive effects of solvent and fuel exposure during aircraft maintenance. *Reprod Toxicol,* 13:155–166.doi:10.1016/S0890-6238(99)00012-X PMID:10378465

192. Rignell-Hydbom A, Axmon A, Lundh T *et al.* (2007). Dietary exposure to methyl mercury and PCB and the associations with semen parameters among Swedish fishermen. *Environ Health,* 6:14.doi:10.1186/1476-069X-6-14 PMID:17488503

193. Krüger T, Spanò M, Long M *et al.* (2008). Xenobiotic activity in serum and sperm chromatin integrity in European and inuit populations. *Mol Reprod Dev,* 75:669–680. doi:10.1002/mrd.20747 PMID:18076054

194. Hernández-Ochoa I, García-Vargas G, López-Carrillo L *et al.* (2005). Low lead environmental exposure alters semen quality and sperm chromatin condensation in northern Mexico. *Reprod Toxicol,* 20:221–228. PMID:15907657

195. Rubes J, Selevan SG, Sram RJ *et al.* (2007). GSTM1 genotype influences the susceptibility of men to sperm DNA damage associated with exposure to air pollution. *Mutat Res,* 625:20–28. PMID:17714740

196. Giwercman A, Rylander L, Rignell-Hydbom A *et al.*; INUENDO (2007). Androgen receptor gene CAG repeat length as a modifier of the association between persistent organohalogen pollutant exposure markers and semen characteristics. *Pharmacogenet Genomics,* 17:391–401.doi:10.1097/01.fpc.0000236329.26551.78 PMID:17502831

197. Cordier S (2008). Evidence for a role of paternal exposures in developmental toxicity. *Basic Clin Pharmacol Toxicol,* 102:176–181.doi:10.1111/j.1742-7843.2007.00162.x PMID:18226072

198. Risk Assessment Forum. Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Report No.: EPA/630/R-03/003F. Washington (DC): U.S. Environmental Protection Agency; 2005.

199. Terrell ML, Small CM, Cameron LL, Marcus M (2008). Comment on "The influence of age at exposure to PBBs on birth outcomes". *Environ Res,* 108:117–120, discussion 121–126.doi:10.1016/j.envres.2008.04.007 PMID:18555987

200. Sweeney AM, Symanski E, del Junco D (2008). Rebuttal to Comment on: "The influence of age at exposure to PBBs on birth outcomes [Environ Res 2007;105(3):370–9]. *Environ Res,* 108:121–126. doi:10.1016/j.envres.2008.04.008

201. Sweeney AM, Symanski E (2007). The influence of age at exposure to PBBs on birth outcomes. *Environ Res,* 105:370–379.doi:10.1016/j.envres.2007.03.006 PMID:17485077

202. Hertz-Picciotto I. Environmental epidemiology. In: Rothman KJ, Greenland S, Lash TL, editors. Modern epidemiology. 3rd ed. Philadelphia (PA): Lippincott Williams & Wilkins; 2008. p. 598–619.

203. Lawson CC, Grajewski B, Daston GP *et al.* (2006). Workgroup report: Implementing a national occupational reproductive research agenda–decade one and beyond. *Environ Health Perspect,* 114:435–441.doi:10.1289/ehp.8458 PMID:16507468

204. Greenland S. Applications of stratified analysis methods. In: Rothman KJ, Greenland S, Lash TL, editors. Modern epidemiology. 3rd ed. Philadelphia (PA): Lipplincott Williams & Wilkins; 2008. p. 283–302.

205. Greenland S. Introduction to regression modeling. In: Rothman KJ, Greenland S, Lash TL, editors. Modern epidemiology. 3rd ed. Philadelphia (PA): Lippincott Williams & Wilkins; 2008. p. 418–55.

206. Ritter L, Arbuckle TE (2007). Can exposure characterization explain concurrence or discordance between toxicology and epidemiology? *Toxicol Sci,* 97:241–252. doi:10.1093/toxsci/kfm005 PMID:17234646

207. Needham LL, Barr DB, Caudill SP *et al.* (2005). Concentrations of environmental chemicals associated with neurodevelopmental effects in U.S. population. *Neurotoxicology,* 26:531–545.doi:10.1016/j.neuro.2004.09.005 PMID:16112319

208. Kang HK, Dalager NA, Needham LL *et al.* (2006). Health status of Army Chemical Corps Vietnam veterans who sprayed defoliant in Vietnam. *Am J Ind Med,* 49:875–884.doi:10.1002/ajim.20385 PMID:17006952

209. Committee to Review the Health Effects in Vietnam Veterans of Exposure to Herbicides, editor. Reproductive and developmental effects. In: Veterans and Agent Orange: Update 2006.Washington (DC): National Academies Press; 2007. p. 517–65.

210. Butler D (2008). Further delays to full Agent Orange study. *Nature*, 452:786–787. doi:10.1038/452786a PMID:18431821

211. Butler D (2005). US abandons health study on Agent Orange. *Nature,* 434:687. doi:10.1038/434687a PMID:15815597

212. Anonymous (2008). A ghost of battles past. *Nature,* 452:781–782 doi:10.1038/452781b.

213. Weinberg CR, Umbach DM (1999). Using pooled exposure assessment to improve efficiency in case-control studies. *Biometrics,* 55:718–726.doi:10.1111/j.0006-341X.1999.00718.x PMID:11314998

214. Faraggi D, Reiser B, Schisterman EF (2003). ROC curve analysis for biomarkers based on pooled assessments. *Stat Med,* 22:2515–2527.doi:10.1002/sim.1418 PMID:12872306

215. Mumford SL, Schisterman EF, Vexler A, Liu A (2006). Pooling biospecimens and limits of detection: effects on ROC curve analysis. *Biostatistics*, 7:585–598.doi:10.1093/biostatistics/kxj027 PMID:16531470

216. Schisterman EF, Perkins NJ, Liu A, Bondell H (2005). Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. *Epidemiology,* 16:73–81.doi:10.1097/01.ede.0000147512.81966.ba PMID:15613948

217. Vexler A, Schisterman EF, Liu A (2008). Estimation of ROC curves based on stably distributed biomarkers subject to measurement error and pooling mixtures. *Stat Med,* 27:280–296.doi:10.1002/sim.3035 PMID:17721905

218. Vexler A, Liu A, Schisterman EF (2006). Efficient design and analysis of biospecimens with measurements subject to detection limit. *Biomed J,* 48:780–791.doi:10.1002/bimj.200610266 PMID:17094343

219. del Junco DJ, Sweeney AM, Paepke O (2006). Paternal blood dioxin, CYP1A1 and neural tube defects in children of Vietnam veterans. *Am J Epidemiol,* 163 Suppl 11;S117–S467.

220. Warner M, Eskenazi B, Patterson DG *et al.* (2005). Dioxin-Like TEQ of women from the Seveso, Italy area by ID-HRGC/HRMS and CALUX. *J Expo Anal Environ Epidemiol,* 15:310–318.doi:10.1038/sj.jea.7500407 PMID:15383834

221. Tsutsumi T, Amakura Y, Nakamura M *et al.* (2003). Validation of the CALUX bioassay for the screening of PCDD/Fs and dioxin-like PCBs in retail fish. *Analyst,* 128:486–492. doi:10.1039/b300339f PMID:12790202

222. Rothman KJ, Greenland S, Poole C, Lash TL. Causation and causal inference. In: Rothman KJ, Greenland S, Lash TL, editors. Modern epidemiology. 3rd ed. Philadelphia (PA): Lippincott Williams & Wilkins; 2008. p. 5–31.

223. Greenland S, Lask TL, Rothman KJ. Concepts of interaction. In: Rothman KJ, Greenland S, Lash TL, editors. Modern epidemiology. 3rd ed. Philadelphia (PA): Lippincott Williams & Wilkins; 2008. p. 71–83.

224. Cox C (2006). Model-based estimation of the attributable risk in case-control and cohort studies. *Stat Methods Med Res,* 15:611–625. doi:10.1177/0962280206071930 PMID:17260927

225. Assmann SF, Hosmer DW, Lemeshow S, Mundt KA (1996). Confidence intervals for measures of interaction. *Epidemiology*, 7:286–290.doi:10.1097/00001648-199605000-00012 PMID:8728443

226. Skrondal A (2003). Interaction as departure from additivity in case-control studies: a cautionary note. *Am J Epidemiol,* 158:251–258.doi:10.1093/aje/kwg113 PMID:12882947

227. Offit PA (2008). Vaccines and autism revisited–the Hannah Poling case. *N Engl J Med,* 358:2089–2091.doi:10.1056/NEJMp0802904 PMID:18480200

228. Scherer M, Trelle S (2008). Opinions on registering trial details: a survey of academic researchers. *BMC Health Serv Res,* 8:18. doi:10.1186/1472-6963-8-18 PMID:18215264

229. Foster MW, Sharp RR (2007). Share and share alike: deciding how to distribute the scientific and social benefits of genomic data. *Nat Rev Genet,* 8:633–639.doi:10.1038/nrg2124 PMID:17607307

230. Moore KL, Persaud TVN. The Developing Human: Clinically Oriented Embryology, 6th ed. Philadelphia: W.B. Saunders, 1998, p. 548