Off to a Good Start: The Influence of Pre- and Periconceptional Exposures, Parental Fertility, and Nutrition on Children's Health

Robert E. Chapin,[1] Wendie A. Robbins,[2] Laura A. Schieve,[3] Anne M. Sweeney,[4] Sonia A. Tabacova,[5] and Kay M. Tomashek[6]

[1]Pfizer Global Research and Development, Safety Sciences, Groton, Connecticut, USA; [2]UCLA Center for Occupational and Environmental Health, University of California at Los Angeles, Los Angeles, California, USA; [3]Division of Reproductive Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [4]Department of Epidemiology and Biostatistics, School of Public Health, Texas A&M University System Health Science Center, Bryan, Texas, USA; [5]National Center for Toxicological Research, U.S. Food and Drug Administration, Rockville, Maryland, USA; [6]Maternal and Infant Health Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

The scientific community is developing a compelling body of evidence that shows the importance of the *in utero* environment (including chemical and hormonal levels) to the ultimate health of the child and even of the aging adult. This article summarizes the evidence that shows this impact begins with conception. Only a full life-cycle evaluation will help us understand these impacts, and only such an understanding will produce logically prioritized mitigation strategies to address the greatest threats first. Clearly, the time for analysis begins when the next generation is but a twinkle in the eye. *Key words:* **birth defects, chemical exposure, conception, fertilization, review.** *Environ Health Perspect* **112:69–78 (2004). doi:10.1289/ehp.6261 available via *http://dx.doi.org/* [Online 24 September 2003]**

There is a growing appreciation of the impact that very early life environment (birth weight, trauma, xenobiotic exposures) has on the health of the child and the adult. The life-course approach to health and disease recognizes that these very early impacts contribute to disease later in life (reviewed in Gillman 2002). From this perspective, any long-term prospective study that attempts to uncover and identify effects on children's health should include an evaluation of the health of both parents prior to and at the time of conception, including xenobiotic load, nutritional state of the mother, and the involvement of any assisted reproductive technologies. These features have already been shown to have demonstrable short-term impacts on the health of the child. Long-term follow-up is essential if we are to fully understand the health burden attributable to these features and to develop more informed hypotheses about exposure–disease relationships. Only from such a complete picture can we hope to make policy decisions that will use our resources most effectively to improve the health of generations to come.

Although by no means an exhaustive review, this article presents some of what we know about these impacts. We conclude that explicit assessment of such exposures and factors before and around the time of conception is indispensable for a real understanding of the determinants of health in our children, and, by extension, the next generation of adults.

The Case for Early Analyses

Explicit measurements of exposure, body burden, or nutritional status in a couple before conception is necessary for numerous reasons.

One critical reason is that recall is variably dependable. Some studies found that the ability of a person to recall specific exposures was good to excellent for some medications (reviewed in Harlow and Linet 1989; Kelly et al. 1990) and some environmental exposures and pesticides (Blair et al. 2001; Feldman et al. 1989) but no better than chance for others (Feldman et al. 1989; Kelly et al. 1990). Although multiple questionnaires may increase the likelihood of obtaining an accurate picture of what the exposures truly were (Farrow et al. 1996), this can be cumbersome and may yield a relatively small increase in confidence.

Additionally, questionnaires have a significant limitation: they capture only what is remembered, which can come only from what the participant knows or is aware of. Importantly, environmental exposures are occult. Exposures to and absorption of excipients and active agents from manufactured products frequently occur without our knowledge or awareness. Phthalates in air or cosmetics can lead to measurable levels *in vivo*, for example (Blount et al. 2000), an internal exposure that no questionnaire, however well constructed and administered, would be able to find and reconstruct. Specific phthalates are capable of disrupting reproductive development in rodents (reviewed in CERHR 2002) and are suspected of causing similar effects in other species, so this gap in our understanding is critical. The limitations of questionnaire data were demonstrated recently for polychlorinated biphenyls (PCBs), where there was no association between potential sources of exposure by questionnaire and actual serum levels of several coplanar PCBs (Shadel et al. 2001). Because few consumers are aware of their exposures or the sources thereof, questionnaire data will not be useful for categorizing study subjects into most-exposed and least-exposed groups for many compounds of current (and probably future) concern.

Arguably, one of the best reasons for performing a prospective longitudinal study is the high probability of finding new associations between previously unanticipated exposures and a health outcome. The more we know about what compounds are in the body and in what amounts, the greater our confidence will be in associating those exposures with health outcomes. This accuracy cannot be acquired with questionnaire proxy data, but requires actual measurements as well as measurements of compounds beyond those that we know today to be hazards.

Making concurrent measurements of body burden is important because of the changing nature of xenobiotics. One of the results of phasing out persistent organic pollutants is that more xenobiotics now have shorter half-lives, and a biological sample taken in late pregnancy or after birth cannot be considered representative of *in vivo* levels at the time of conception or during early pregnancy (reviewed in MacIntosh et al. 1999). An excellent example is methoxychlor, developed to replace dichlorodiphenyltrichloroethane (DDT). It has a similar spectrum of intended effects but is much more readily excreted and thus has a much-reduced tendency to bioaccumulate (Kapoor et al.

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Address correspondence to R. Chapin, Pfizer Global R&D, Safety Sciences, Eastern Point Rd., MS8274-1336, Groton, CT 06340 USA. Telephone: (860) 441-0571. Fax: (860) 715-3577. E-mail: robert_e_chapin@groton.pfizer.com

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1970). Phthalates are another example: these are cleared from the body with relative speed (several days to a few weeks) and do not bioaccumulate significantly (Tanaka et al. 1978). From the public health and environmental perspectives, this is incontrovertibly good, although it means that actually determining which levels of which compounds are adverse requires repeated biological sampling and analysis.

A corollary of these short half-lives is considerable sample-to-sample variation. Even with no obvious changes in behavior, urinary levels of some pesticides have been shown to vary by factors of four times or more (MacIntosh et al. 1999). Consequently, a single sample will be poorly representative of the long-term levels in that individual.

A benefit of repeated sampling is that it would accommodate the altered behaviors exhibited by women when they know they are pregnant (Lelong et al. 2001; Root and Browner 2001). If these altered behaviors also reduced their exposures to, and internal burdens of, harmful xenobiotics, then the reduced internal half-lives plus the altered behaviors will significantly reduce internal levels, making late-stage analyses even less representative of earlier levels. Measuring internal levels of xenobiotics around the time of conception will provide a much more realistic picture of the exposure of the embryo at the critical period of conception.

Study of the nutritional status of a woman prior to pregnancy would allow for the timely measurement of prepregnancy weight and the collection of dietary history and laboratory data. Studies have found that self-reported prepregnancy weight may be biased, especially among overweight women, who are more likely to underestimate their prepregnancy weight (Perry et al. 1995; Stevens-Simon et al. 1986). Dietary deficiencies, thought to be due to long-standing dietary habits and not a sudden insult, may resolve if a woman changes her diet after she knows that she is pregnant. By getting a dietary history prior to conception, we can eliminate recall bias and document diet during the critical periods (Sempos 1992; Wei et al. 1999). Early collection of laboratory data is important for several reasons. First, hemodilution, which begins early in the second trimester and varies in degree and timing among individuals, makes interpretation of a number of micronutrient levels difficult (e.g., plasma zinc concentrations). Second, other physiologic changes that occur during pregnancy (e.g., increased renal excretion of water-soluble vitamins, hormone-mediated transfer of nutrients to placenta and mammary glands, decrease of serum albumin) make determining micronutrient status during pregnancy difficult (King 2000). Third, it may be important to measure micronutrient levels prior to pregnancy because changes in behavior (e.g., cessation of smoking and/or alcohol consumption) and use of iron and multivitamin supplements during pregnancy may affect laboratory values. Last, changes in the type and quantity of food consumed as well as changes in physical activity may affect values (King 2000). By evaluating nutritional status prior to pregnancy or in early pregnancy prior to physiologic and behavioral changes, one can more accurately evaluate a woman's nutritional status and the associations between nutritional status during early pregnancy and subsequent functional outcomes. Knowledge of such associations could lead to interventions that prevent adverse outcomes, such as the provision of folic acid to prevent birth defects.

The rest of this review will focus on a variety of key issues and their relationships to the health of the resultant child: compounds in semen; compounds in the prepregnant female; the effects of assisted reproductive technologies (ARTs); the effects of preimplantation exposures; the influence of early maternal nutrition; and a review of the period of greatest sensitivity for producing malformations and fetal alterations.

Compounds in Semen

The pathway through which environmental toxicants contaminate semen and affect offspring is diagrammed in Figure 1. In this scenario, a male is exposed to an environmental toxicant that is absorbed, distributed, biotransformed, and excreted as metabolite or parent compound in seminal fluid. Alternatively, compounds can be adsorbed to sperm and be introduced directly into the egg at fertilization [e.g., cocaine or cyclophosphamide (reviewed in Hales and Robaire 2001; Yazigi et al. 1991)]. Any contaminants in semen are transmitted to a woman in the ejaculate. The contaminant is absorbed by the female, where it may reach and adversely affect a current pregnancy and perhaps remain in the woman's body to influence future pregnancies, or it enters the egg with the sperm and provides an initial direct dosing of the conceptus.

Teratogenic, carcinogenic, and endocrine-disrupting agents have all been detected in human seminal fluid. For example, pesticide residues including the dioxin congener 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, hexachlorocyclohexane isomers (as prevalent as 100% in some populations tested), DDT and its metabolites, and 2,4-dichlorophenoxyacetic acid have been identified and quantified in seminal plasma (Arbuckle et al. 1999; Kumar et al. 2000; Pflieger-Bruss and Schill 2000; Schecter et al. 1996; Schlebusch et al. 1989; Stachel et al. 1989; Szymczynski and Waliszewski 1981; Wagner et al. 1990). Metals such as lead and cadmium, both known developmental toxicants, have been measured in human seminal fluid by a number of research groups (Dawson et al. 2000; Kumar et al. 2000; Noack-Fuller et al. 1993; Saaranen et al. 1989; Stachel et al. 1989; Telisman et al. 2000; Xu et al. 1993). The organic solvents benzene, toluene, and xylene can be found in semen (Xiao et al. 1999), and ethanol can be detected, entering through simple diffusion (Luke and Coffey 1994). Finally, seminal fluid of smokers contains nicotine, its metabolites, aromatic hydrocarbons, and precursors of mutagenic nitrosamines (Hoffman et al. 1994; Pacifici et al. 1993; Rivrud 1988; Wong et al. 2000). The critical question of dose sufficiency (are these compounds present at a level sufficient to cause adverse effects?) remains unanswered.

The composition of seminal fluid changes normally as a function of frequency of ejaculation, hydration, nutritional state, and exposures to the male. Because of this variability, it would be necessary to monitor the fluid in a prospective way to make clear associations between contaminants present in seminal fluid (and transmitted to a female partner) and any abnormalities in the offspring. Only recently have laboratories been capable of measuring multiple low-concentration contaminants in ever-smaller amounts of seminal fluid. This capability presents a significant opportunity. A large longitudinal study, coupled with advances in detection and measurement of chronic, low-level contaminants in seminal fluid, would provide an excellent opportunity to more clearly delineate any associations between semen-mediated very early exposures and health outcomes in the offspring.

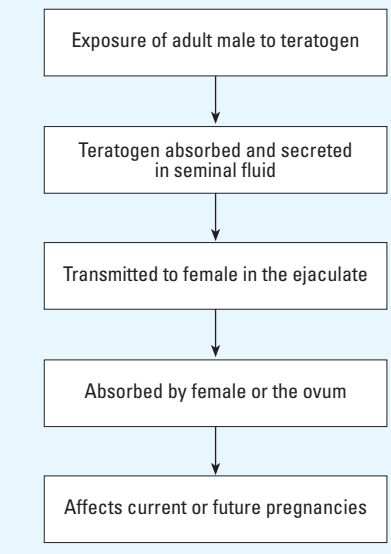

Figure 1. Schematic of possible pathway of exogenous agents that could directly affect the conceptus via nonsperm contributions from the male.

Altered Semen Quality

An important area often neglected when evaluating environmental exposures and children's health relates to sperm DNA–derived effects. Exposures to the father that cause germline DNA or chromatin damage and consequently affect offspring health are well documented in animal models (reviewed in Hales and Robaire 2001). Similar studies in humans (exposing the male to toxicants, mating over time, monitoring offspring for adverse effects) cannot even be approached for obvious ethical reasons. Yet, there is undisputed evidence that the human male significantly affects the health of his children through sperm DNA in paternally mediated Mendelian genetic defects, chromosomal aberrations (e.g., paternal translocation carriers), and aneuploid sperm (associated with 30–50% of Klinefelter, 80% of Turner, 5% of Down, and 100% of extra Y chromosome syndromes) (Blanco et al. 1998; Eskenazi et al. 2002; Hassold 1998; Hixon et al. 1998; Martinez-Pasarell et al. 1999; Pangalos et al. 1992). Researchers in ART clinical settings have shown that both paternal sperm chromatin and DNA quality affect pre- and postimplantation development of offspring (Evenson et al. 2002; Hammadeh et al. 1996; Tesarik et al. 2002). It is evident that sperm with abnormalities of DNA and/or chromatin are able to fertilize and transmit abnormal DNA to the conceptus. It is also evident from numerous studies that environmental exposures can induce mutations, chromosomal aberrations, chromatin, or epigenetic protein changes in human sperm (selected examples in Table 1). This leads to a model for environmentally induced male-mediated effects on offspring, as depicted in Figure 2. This model clearly demonstrates the importance of a study design that monitors environmental exposures to the father during sperm development and maturation prior to and at the time of fertilization. Because sperm are constantly being replenished in the male genital tract, only prospective study designs are able to capture direct effects of environmental exposures on sperm DNA quality at the time of conception. Previous studies that have tried to establish cause–effect relationships by evaluating DNA quality of sperm after the birth of an abnormal child have not been successful because of this temporal flaw. Recent advances in molecular-cell and genetic laboratory techniques now allow sophisticated assessment of human sperm DNA and chromatin damage (Evenson et al. 2002; Perreault et al. 2000, 2003). There are new understandings of the critical role of sperm chromatin organization and paternal DNA in early embryo development (Evenson 1999; Sakkas et al. 1998; Tesarik et al. 2002). A prospective longitudinal study would be the most definitive way to finally answer the question of dose, timing, and types of exposures that cause specific sperm DNA and chromatin damage that affects offspring health.

Compounds in the Female

In addition to the father's contribution, the mother brings a time-averaged and complex body burden of xenobiotics to the conception and the early pregnant period.

What is known about the effects of compounds at this time is recent and relatively limited. This is partly because earlier studies examining the impact of these exposures in the environmental setting and reproductive effects were severely hampered by crude measures of exposure. Lack of biological assays to measure many of these contaminants in tissue specimens, the high cost of using these assays in large epidemiologic studies when they were available, and challenges related to the interpretation of the results when exposures were measured after the adverse reproductive event occurred limit the conclusions that can be drawn from these investigations. Rapid advancements in laboratory techniques, including biological assays as screening methods for exposure to these contaminants, as well as highly sensitive techniques to measure specific congeners of the compounds or their metabolites at reasonable costs are exciting developments that hold great promise in enhancing our understanding of the impact of these exposures on reproductive events (Humphrey et al. 2001; Seidel et al., 2000).

Despite these impediments, data do exist from national studies to describe general population exposures to some of these contaminants. The National Health and Nutrition Examination Survey II (NHANES II) measured serum levels of selected pesticides in approximately 21,000 participants ranging in age from 12 to 74 years (Murphy and Harvey 1985). Increasing age was related to the presence of detectable levels of DDT, with the proportion exposed ranging from 14 to 51% across the age spectrum. Serum values ranged from 2 to 58 ppb. Detectable levels of the DDT metabolite *p,p′*-DDE were found in serum in nearly 99% of the population, with values ranging from 1 to 378 ppb, which also increased with age.

Although the use of DDT in the United States has been banned for more than 30 years, exposure may continue through consumption of contaminated foods, including products imported from countries where DDT is still being used, as well as foods contaminated because of the environmental persistence of DDT. Additional data on serum DDE levels in selected subgroups from the U.S. population are available from several investigations that were conducted to examine the effect of DDE and PCB exposure and risk of breast cancer. A recent study involved the pooling of five U.S. studies that included women over an interval spanning 1974–1997 (Laden et al. 2001). Median values for serum DDE levels ranged from 0.41 to 1.67 μg/g lipids. Several studies have also assessed DDE levels among consumers of sport-caught fish

Table 1. Evidence that selected environmental exposures are associated with DNA, chromatin, or epigenetic damage in human sperm.

Selected environmental exposures	Genetic or epigenetic damage in human sperm	Author/year
Organophosphate pesticides	Aneuploidy	Recio et al. 2001 Padungtod et al. 1999
Tobacco smoke	DNA strand breaks[a]	Potts et al. 1999
	Acid-labile DNA sites	Potts et al. 1999
	Benzo[*a*]pyrene diol epoxide adducts	Zenzes et al. 1999
	8-Hydroxydeoxyguanosine	Fraga et al. 1996
	Aneuploidy	Robbins et al. 1997 Rubes et al. 1998 Harkonen et al. 1999
Air pollution	Aneuploidy	Robbins et al. 1999
	Acid-labile DNA sites	Selevan et al. 2000
1,3-Butadiene metabolites	DNA strand breaks	Anderson et al. 1997
Ethylene glycol monomethyl ether	DNA strand breaks	Anderson et al. 1997
Lead	DNA strand breaks	Anderson et al. 1997

[a]Sergerie et al. (2000) did not find an association between smoking and DNA strand breakage.

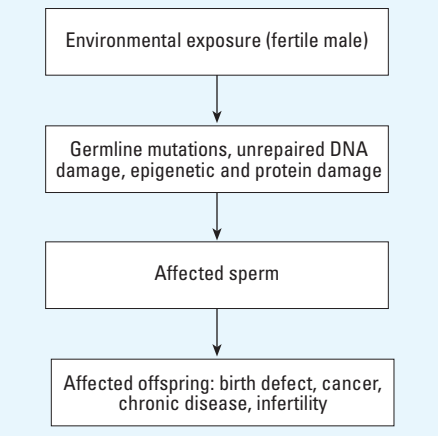

Figure 2. Schematic of pathway of sperm-mediated effects on offspring health and development.

from contaminated waters (Hanrahan et al. 1999; Schantz et al. 2001). These reports indicate that consumers of sport-caught fish have higher serum DDE levels compared with those of nonconsumers (Hanrahan et al. 1999; Schantz et al. 2001).

These same studies in consumers of sport-caught fish versus nonconsumers have provided data on serum PCB levels among consumers compared with non–fish-eaters. Background PCB levels in the U.S. population were reported to be 4–8 ppb in the early 1980s (Kreiss 1985) and is currently 0.9–2.2 ppb (ATSDR 2000). In the Hanrahan study conducted in 1993–1994, male fish-eaters had a mean serum PCB level of 4.8 ppb compared with 1.5 ppb among their nonconsuming counterparts (Hanrahan et al. 1999), whereas female fish-eaters had levels of 2.1 ppb versus 0.9 ppb among female non–fish-eaters. Participants in the Michigan fish-eaters cohort, which consists of individuals who were consuming ≥ 26 pounds of sport-caught fish per year (fish-eaters) and those eating < 6 pounds per year (non–fish-eaters), when enrolled in 1980, had considerably higher serum PCB levels (Tee et al. 2003).

How do these maternal burdens impact the developing fetus or child? In a cohort of infants born to Michigan women in 1983 that included consumers of sport-caught fish and those who ate fish other than sport caught, significantly lower birth weight and delayed motor reflexes were noted among exposed infants at birth (Fein et al. 1984). Overall, mean PCB levels in cord blood PCB in this study were 3 ng/mL (± 2 ng/mL), and in maternal serum 6 ng/mL (± 4 ng/mL). Importantly, persistent effects on cognitive functioning among the exposed children were reported in a follow-up at 11 years of age (Jacobson and Jacobson 1996). This increased burden tends to weigh most heavily on lower socioeconomic minority populations (Sweeney et al. Unpublished data), and these increased exposures bring adverse consequences. Only by determining with certainty the different exposures during development and following many outcomes postnatally can we ascertain the full burden of these exposures, which is the first step toward their elimination.

Other examples of endocrine-active compounds (EACs) that have been detected in serum and adipose tissue samples obtained from representative populations from the United States include beta-benzene hexachloride, dieldrin, *trans*-nonachlor, hexachlorobenzene, heptachlor epoxide, and oxychlordane (Murphy and Harvey 1985). Another class of EACs measured in NHANES III, the phthalates, has generated considerable concern regarding potential adverse reproductive effects. Using a novel and highly selective technique, investigators measured monoester metabolites of seven commonly used phthalates in 289 adults from the NHANES III study population (Blount et al. 2000). An important finding of this study was the significantly higher urinary levels of monobutyl phthalate among women of reproductive age compared with those of other age/gender groups. In addition, rural women of childbearing age had higher levels of benzyl butyl phthalate compared with those of older rural women. This is of concern because both of these phthalate isomers have been implicated in animal studies of reproductive toxicity (Gray 1998; Jobling et al. 1995; Shiota et al. 1980; Wilkinson and Lamb 1999). Human studies are scarce, but a recently published case–control study examined the association between premature thelarche (premature breast development) and phthalate exposure among girls 6 months to 8 years of age (Colon et al. 2000). With serum as the medium, significantly higher levels of dimethyl, diethyl, dibutyl, and di-2-ethylhexyl phthalate (DEHP) were measured in the cases. With regard to DEHP, the ratio of average concentrations between control:case samples was 70:450 ppb. Animal studies show that DEHP has its greatest effects while the reproductive system is forming (within the first 4 weeks in a human pregnancy), so identifying and quantifying phthalate levels prepregnancy will be particularly important in ensuring that all the impacts on reproductive development are accounted for and quantified.

Finally, although not classified as EACs, lead and mercury are important environmental contaminants associated with adverse effects on reproduction. Although an elevated lead level in children is defined as > 10 µg/dL by the Centers for Disease Control and Prevention (CDC 2000), a "safe" lead level remains controversial (Sanborn et al. 2002). As with PCB levels, blood lead levels in the United States have been steadily declining. However, subgroups, particularly low-income populations and children requiring social services and foster care, remain at high risk for elevated blood lead levels (Chung et al. 2001).

Organic mercury, which is mostly methyl mercury, bioaccumulates in the aquatic food chain and can be present in relatively large amounts in fish living in contaminated waters. Effects on the developing nervous system have been well documented in studies following the Minamata Bay and Iraq poisoning incidents (reviewed in CDC 2000), as well as in more recent studies of children exposed prenatally at much lower levels (Grandjean et al. 1998). A recent report issued by the National Research Council estimates that 60,000 infants per year in the United States may be prenatally exposed to levels of methyl mercury sufficient to cause neurological and cognitive impairments (National Research Council 2000).

Concern has also focused (Toppari et al. 1996) on the possible role of endocrine-disrupting chemicals with either estrogenic or antiandrogenic effects in the marked rise (almost doubling) in the prevalence of hypospadias in the United States and Europe since the late 1960s (Kallen et al. 1986; Paulozzi et al. 1997). Because the development of the male genital organs is under hormonal control, the main hypotheses as to the etiology of hypospadias are centered on either disturbance of endogenous hormonal production or exposure to exogenous hormones (Dolk 1998). The hypothesis is supported by the most recent findings of an increased risk of hypospadias in sons of women who themselves had been prenatally exposed to diethylstilbestrol (DES; a synthetic estrogen widely prescribed to pregnant women between 1938 and 1975 to treat habitual abortions) (Klip et al. 2002). This transgenerational effect was associated with maternal *in utero* exposures to DES early in the first trimester. A link with cryptorchidism has also been noted. Because the reproductive system forms early in the first trimester, obtaining the best possible estimates of exposure during that time will help us determine the degree of environmental contribution to these problems.

Early first-trimester drug exposures have been linked to birth defects. Recent examples are the illicit use of amphetamines associated with significantly increased risk of predominantly cardiovascular and musculoskeletal malformations (McElhatton et al. 1999), and the misuse of misoprostol, a synthetic analog of prostaglandin E, resulting in limb deformities attributable to vascular disruption (Gonzales et al. 1998).

Adverse developmental outcomes have also been associated with early exposures to environmental physical factors. Pre- and periconceptional parental occupational exposures to low-level ionizing radiation have been repeatedly associated with increased risk of fetal loss and neural tube defects (NTDs) (Doyle et al. 2000; Inskip 1999; Sever et al. 1988).

Given the findings above and the recent quantitation of a number of xenobiotics in human ovarian follicular fluid (Younglai et al. 2002), the impact of these environmental chemicals on human reproduction has become a high priority for public health researchers (Barlow et al. 1999). A paper describing the conclusions reached by the recent U.S. Environmental Protection Agency–sponsored "Workshop to Identify Critical Windows of Exposure for Children's Health" called for research into the effects of environmental exposures during the periconceptional and prenatal periods on reproductive and children's health (Pryor et al. 2000). With advances in laboratory techniques and statistical modeling approaches for exposure assessment, we are

now positioned to be able to quantify the degree of exposure that occurs early, which will affect strategies for preventive health policies in the future.

Assisted Reproductive Technologies

The evolution of and increasing use of various ARTs for the treatment of infertility has provided additional insight into the importance of considering exposures that occur very early in conception or even just prior to conception. ART encompasses those procedures in which both eggs and sperm are handled in the laboratory. To date, a range of effects have been reported in association with various facets of ART procedures. Some effects can be linked to specific exposures that occur in conjunction with a particular step in the ART cycle; for other effects, an association with ART is suggested, but it is not possible from available data to disentangle the specific exposures or mechanisms. Nonetheless, the data accumulating from ART pregnancies underscore the importance of periconceptional exposures.

The vast majority of ART procedures include ovarian stimulation with either clomiphene citrate, human pituitary gonadotropins (various formulations), or both. Several potential adverse effects have been suggested from studies focusing on the use of these ovulation-stimulating drugs. Perhaps the most striking finding is the association reported between artificial induction of ovulation and monozygotic (MZ) twinning. Unlike dizygotic twinning, the prevalence of MZ twinning has been remarkably constant worldwide (Dunn and Macfarlane 1996; Guttmacher 1953; Hur et al. 1995; MacGillivray 1986; Parazzini et al. 1991). However, in the late 1980s, Derom and colleagues reported a 2-fold increase in the MZ twin birth rate among women from their population-based study in East Flanders, Belgium, who had conceived after therapy with an ovulation-inducing medication, compared with the rate among women who conceived naturally (Derom et al. 1987). This was the first report that an extrinsic factor might affect zygotic division. Because twinning carries a suite of increased health risks (Kovacs et al. 1989; Naeye et al. 1978), this is not viewed as a benign outcome. Use of ovulation drugs, particularly clomiphene, has also been implicated as a risk factor for spontaneous abortion (Balen et al. 1993; Dickey et al. 1996; McFaul et al. 1993; Oktay et al. 2000; Shoham et al. 1991), preterm delivery (Olivennes et al. 1993; Sundstrom et al. 1997) and low birth weight (Olivennes et al. 1993; Sundstrom et al. 1997). This research raises the question about whether such medications might adversely affect oocytes directly. Studies in mice suggest that clomiphene might affect oocyte maturation and rates of aneuploidy (London et al. 2000).

Perhaps the most obvious source of unique exposures linked to ART pregnancies is the culture media in which preimplantation embryos are fertilized and cultured. A body of both animal and human research is accumulating that implicates several factors in culture media as having an effect on embryo development. High concentrations of glucose in culture media have been associated with decreased embryo quality, defined by cleavage rates, degree of fragmentation, and blastocyst development (Coates et al. 1999; Gardner et al. 2000). These effects were reported from studies of human embryos and embryos from several other mammalian species and may be mediated by altered intracellular pH (Bavistor 1999). Mouse embryos incubated in serum-containing media had altered cleavage rates compared with the rates in embryos cultured in media without serum (Khosla et al. 2001). Moreover, the addition of serum to culture media has been linked to effects on DNA methylation and deregulation of imprinted genes. These findings have implications for outcomes associated with imprinted genes, such as those controlling fetal growth. Finally, several case reports and studies have suggested a general association between *in vitro* culture to the blastocyst stage and MZ twinning (Behr et al. 2000; da Costa et al. 2001; Gorrill et al. 2001; Peramo et al. 1999; Sheiner et al. 2001; Van Langendonckt et al. 2000).

ART embryos might also be affected by various techniques in which the embryos are manipulated beyond *in vitro* culture. In intracytoplasmic sperm injection (ICSI), a single spermatozoon is directly injected into the egg through the oocyte membrane. Studies on embryos from various mammalian species suggest that ICSI is related to a host of chromosomal abnormalities including effects on spindle apparatus, microtubules, chromatin behavior, cell cycle checkpoints, and chromosome positioning (Hewitson et al. 1999; Luetjens et al. 1999; Ramalho-Santos et al. 2000; Sutovsky et al. 1996; Terada et al. 2000). Increases in both de novo sex chromosome abnormalities and de novo autosomal rearrangements have been reported among infants conceived using ICSI (Bonduelle et al. 2002). The other, longer-term health changes associated with this procedure have yet to be evaluated.

Another embryo manipulation technique that has raised concern is "assisted hatching" (Alikani et al. 1994; Nijs et al. 1993; Skupski et al. 1995; Slotnick and Ortega 1996;). Comparative studies have documented an increased risk for MZ twinning in ART pregnancies that included assisted hatching (Hershlag et al. 1999; Schieve et al. 2000), and animal studies have shown that MZ twinning can be induced through mechanical or chemical manipulation of the zona pellucida (Allen and Pashen 1984; Picard et al. 1985; Sotomaru et al. 1998; Talansky and Gordon 1988).

Infant outcomes have also been linked to ART generally. ART is recognized as an important contributor to the U.S. low birth weight rate because of the known associations between ART and multiple birth (CDC 2002a, 2002b) and between multiple birth and low birth weight (Martin and Park 1999). Additionally, studies have suggested that low birth weight rates are increased among singleton infants conceived with ART compared with naturally conceived infants or population-based rates (Bergh et al. 1999; Dhont et al. 1999; FIVNAT 1995; Friedler et al. 1992; Gissler et al. 1995; MRC Working Party 1990; Schieve et al. 2002; Verlaenen et al. 1995; Westergaard et al. 1999). Additional analysis suggests that the increased risk for term low birth weight may be related to the ART procedure itself rather than the underlying infertility.

Equivocal results have been reported for the association between ART and birth defects in the offspring (Bergh et al. 1999; Bonduelle et al. 2002; Dhont et al. 1999; Ericson and Kallen 2001; FIVNAT 1995; Friedler et al. 1992; Hansen et al. 2002; MRC Working Party 1990; Verlaenen et al. 1995; Wennerholm et al. 2000; Westergaard et al. 1999). Studies to date have suffered from various methodological problems, including low statistical power, particularly to assess individual defects separately, and differential case ascertainment and coding schemes for infants conceived using ART and infants conceived naturally. Nearly all studies relied on retrospective registry data. Two recent studies have shown increased risk for various birth defects among ART-conceived infants (NTDs, alimentary tract atresia, omphalocele, hypospadias, and cardiovascular, urogenital, chromosomal, and musculoskeletal defects) (Ericson and Kallen 2001; Hansen et al. 2002). These studies are particularly noteworthy because they demonstrated elevated risks even among singleton infants. A longitudinal prospective study that looks at long-term outcomes would be immensely valuable in delineating yet-undiscovered associations between long-term health and method of conception. These concerns are made more acute by a recent study that reported an increased risk for developmental delay and cerebral palsy among children conceived with ART (Stromberg et al. 2002). These effects remained elevated when analyses were limited to singleton births, although the study suffered from a number of methodological drawbacks, including a lack of statistical power to adequately assess subgroup findings.

Collectively, these data show that ART methods have health consequences for the offspring. Most of these studies have been

relatively short-term and have limited power to evaluate correlations between early procedures and exposures, and later health outcomes. Given that this much is already known about health effects of ART, we expect that more relationships would be found if we evaluated health over a longer term. A large longitudinal study would be uniquely suited to define these relationships and would help prospective parents to make fully informed decisions about the true costs and burdens of ART methods.

Preimplantation and Early Gestational Exposures

Implantation in humans is a gradual process that takes up most of the second week of pregnancy (reviewed in O'Rahilly and Muller 2001). In rats and mice, implantation occurs between days 4 and 6 (reviewed in Cummings 1993). Although organogenesis was believed to be the most vulnerable time for inducing terata (see below), some recent studies have found that preimplantation exposures can have serious impacts on fetal health.

The first example of this, ethylene oxide, was reported by Generoso et al. (1987). Subsequently, that same group reported that a number of chemicals are potent teratogens when administered to mice 1–6 hr after mating but are ineffective either before or after that window (reviewed in Rutledge et al. 1992). In addition, the microtubule disruptor nocodazole reportedly can disrupt midgestation development when administered only at the time of sperm entry (Generoso et al. 1989). The authors conclude that there are primarily epigenetic mechanisms at work.

Subsequently, cyclophosphamide, actinomycin D, methyl mercury, alkylating agents, cross-linkers, and direct and indirect mutagens have all been found to cause malformations or growth alterations in offspring when administered to pregnant rodents prior to implantation (Iannaccone et al. 1987; reviewed in Rutledge et al. 1992).

Less extreme exposures have effects in humans. Parental periconceptional smoking, for example, was associated with a significant decrease in offspring male:female ratio. In a study on 11,815 singleton infants delivered in Japan (Fukuda et al. 2002), the daily parental consumption of cigarettes during the periconceptional period (from 3 months before the last menstruation to when the pregnancy was confirmed) was associated with a significant decline in the neonatal male:female ratio.

This study fits into the larger picture of the male:female newborn ratio decline in a number of industrialized countries during the past decades, including Denmark, the Netherlands, Norway, Finland, Sweden, Germany, Canada, and the United States (Davis et al. 1998). The reason for this reduction is not clear, but it has been suggested that chronic exposures to toxic environmental agents that predominantly affect males and the male reproductive system could lead to a lower male:female ratio, as seen, for example, after the exposures to dioxins in Seveso, Italy (Mocarelli et al. 2000), PCBs and dibenzofurans in Taiwan (Rogan et al. 1999), DBCP in Israel (Potashnik et al. 1984), medical radiation (Hama et al. 2001), and methyl mercury in Minamata Bay (Sakamoto et al. 2001). Evidence has emerged that mammalian sex ratio at birth is partially controlled by parental hormone levels at the time of conception (James 1996). These levels could be altered by exposures to endocrine-active substances such as exogenous estrogens or by compounds that interfere with a normal hormone's actions. Thus, the raised concentration of human chorionic gonadotrophin during the first trimester of pregnancies complicated by hyperemesis has been implicated as a reason for the lower male:female ratio found in such pregnancies compared with normal ones (Sorensen 2000). Although these birth ratios may normalize with time, if the sex ratios are related to hormone levels at conception, they may one day be considered a biomarker of internal hormone status. A large prospective study measuring hormone levels and postnatal health (as well as gender) will be one way to test this hypothesis.

It is plausible that some proportion of the adverse health effects seen in some children could be the result of yet-to-be-recognized preimplantation exposures. Only by actually determining what's "on board" the mother around the time of conception and implantation can we identify those influences.

Maternal Nutritional Status

Maternal nutrition is thought to be an underlying determinant of a variety of pregnancy outcomes. A growing body of literature has established the association between maternal nutritional status and adverse pregnancy outcomes (Luke 1994a, 1994b; Ramakrishnan et al. 1999). Most studies have evaluated women well after the pregnancy has been established and the woman knows she is pregnant, typically during the second or third trimester. In contrast, the effect of a woman's nutritional status prior to pregnancy and during the first trimester on pregnancy outcomes has been rarely studied, even though evidence suggests that this is a vulnerable period in embryo and fetal development (Ashworth and Antipatis 2001; Czeizel 1995; Robinson et al. 1999). Studying women's nutritional status during this period will help determine the impact of early nutritional deficiencies or excesses on birth outcomes, and thereby assist in prevention efforts by enabling the design of more timely interventions.

Pregnancy is a time of increased cellular metabolism and rapid growth and development; therefore, there is an increased demand for energy and nutrients during pregnancy (King 2000). Changes in nutritional metabolism are driven by hormonal changes, fetal demands, and maternal nutritional supply. In general, well-nourished women need only a small amount of additional energy because of physiologic adaptations such as a lowered basal metabolic rate and reduced physical activity. However, these adaptations are limited in their capacity to adjust to changes in nutritional metabolism such that maternal undernutrition or micronutrient deficiencies or excesses during a critical stage of development may compromise fetal growth and development.

Nutritional requirements vary over the course of the pregnancy, depending on the stage of prenatal development. A woman's nutritional status prior to pregnancy, measured by prepregnancy weight, has been significantly associated with intrauterine growth retardation (weight for gestational age at birth < 10th percentile of a reference population) and low birth weight (< 2.5 kg) (WHO 1995). Higher maternal body mass index before pregnancy has been associated with a lower risk of having a small-for-gestational-age infant and an increased risk of late fetal death and NTDs (Cnattingius et al. 1998; Werler et al. 1996). Improving women's nutritional status in the months prior to pregnancy has resulted in higher birth weights and lengths (Caan et al. 1987). Data from the Collaborative Perinatal Project, a study of more than 55,000 pregnancies, found that maternal prepregnancy weight and gestational weight gain act independently of each other but are additive in their effect on fetal growth (Luke 1994b). It has been hypothesized that poor periconceptional nutrition may adversely affect placental growth, leading to insufficient fetoplacental exchange of nutrients, or that underlying micronutrient deficiency such as iron deficiency or anemia may stimulate the hypothalamic–pituitary–adrenal axis, thereby affecting early growth and length of gestation (Allen 2001; Robinson et al. 1995). A better understanding of the effects of preconceptional nutritional deficits (or excesses) and their causal mechanisms could lead to more effective interventions to reduce these fetal exposures and could help separate xenobiotic etiologies from nutritional causes of ill health.

Although nutrient requirements during early pregnancy are quantitatively small, specific nutritional deficiencies and toxicities can have adverse effects on prenatal development. During the first 2 weeks postovulation, maternal protein deficiency is associated with a variety of adverse pregnancy outcomes in animal studies (Desai et al. 1996; Gressens et al. 1997; Langley-Evans 2000). One such

study found that offspring of female rats fed a low protein diet during preimplantation and then a normal control diet for the remainder of pregnancy had lower birth weights, increased growth rate postweaning, increased systolic blood pressure, and disproportionate growth of the liver and kidneys compared with control offspring (Kwong et al. 2000). Much of what we know about the importance of preimplantation nutrition in humans we have learned from human *in vitro* fertilization studies that suggest that certain amino acids (among other essential nutrients) are necessary substrates for the development and viability of the conceptus posttransfer (Conaghan et al. 1998; Devreker et al. 2001). Although evidence from human epidemiologic studies is not sufficient to conclude that there is a relationship between low protein intake in pregnancy and pregnancy outcomes (Metges 2001), studies of pregnant women exposed to extreme food deprivation suggest that this may be a vulnerable period in human development as well (Susser and Stein 1994). Specifically, women deprived during the 3-week period beginning after menstruation through implantation were more likely to have infertility problems and/or an infant born with an NTD. Those exposed *in utero* were more likely to have schizophrenia and schizoid and antisocial personality at 19 years of age.

During embryogenesis, the period of development from the third through eighth postovulatory weeks, an inappropriate dietary supply, a functional deficiency, or an excess amount of a micronutrient can be teratogenic. Although the causal mechanisms are not well understood in some cases, micronutrients are integral components of cellular metabolism. As such, micronutrients have the potential to impair cellular growth and replication in the rapidly growing embryo and fetus. Perhaps the best-known example of the impact of a micronutrient during this vulnerable period of development is the relation between folic acid deficiency and NTDs. In the last decade, periconceptional folic acid supplementation has been shown to prevent the occurrence and recurrence of NTDs (Lumley et al. 2002). NTDs occur within the first month after conception and are thought to be caused by failure of the neural tube to close (Sadler 1998). However, the mechanism by which folic acid prevents NTDs is unknown, and folic acid does not prevent all NTDs. Studies have suggested that the etiology of NTDs may be multifactorial, and deficiencies in other nutrients such as zinc, methionine, vitamin B12, and selenium may be involved (Guvenc et al. 1995; Kirke et al. 1993; Shoob et al. 2001; Velie et al. 1999). Excessive levels of micronutrients have also been associated with NTDs (e.g., vitamin A) (Rothman et al. 1995). In addition, there is increasing evidence that inadequate micronutrient status during organogenesis may be associated with an increased risk for other congenital anomalies such as orofacial clefts, conotruncal cardiac defects, and defects of the urinary system, as evidenced by reduced risk of these anomalies with periconceptional multivitamin supplementation (Botto et al. 1996; Czeizel 1996; Hall and Solehdin 1998; Itikala et al. 2001).

Micronutrient deficiencies have also been associated with adverse pregnancy outcomes such as preterm delivery (gestation < 37 weeks) and low birth weight. A recent review found an association between maternal anemia (i.e., low hemoglobin concentration) and these outcomes (Rasmussen 2001), but the majority of these studies document only the effect of second-trimester anemia. A few studies have found an association between first-trimester anemia and preterm delivery (Scanlon et al. 2000; Scholl and Reilly 2000; Zhou et al. 1998). Although anemia is the most commonly used indicator for iron deficiency, iron deficiency is rarely assessed in pregnancy, and anemia is neither a sensitive nor specific indicator for iron deficiency. Some evidence suggests that iron deficiency anemia is associated with an increased risk for preterm delivery (Lu et al. 1991; Scholl et al. 1992), though this link is still unconfirmed (Rasmussen 2001). There is also evidence that deficiencies of other micronutrients such as zinc, calcium, and folic acid may be associated with preterm delivery and low birth weight.

Research into the area of periconceptional nutrition has shown that maternal nutritional deficiencies and toxicities in early pregnancy can have irreversible effects. Greater emphasis on research during the periconceptional period may yield data that confirm that other nutritional deficiencies or toxicities, previously unstudied in human populations, may affect health outcomes in the fetus, infant, and child. For a better understanding of the role of periconceptional nutrition on the health of the pregnant mother, fetus, and ultimately the infant, a woman's nutritional status should be evaluated during this critical time of development. Because women often do not know that they are pregnant until after the period of organogenesis, it is critical that any long-term study of health outcomes in children and adolescents enroll the woman before she becomes pregnant.

This review has focused on environmental exposures, although there are clearly other factors that were not considered, and which we know impact pregnancy outcome and the child's health, including a large number of diseases (reviewed in Geist and Koren 2001) and environmental heat (Wells and Cole 2002).

Conclusions

Even this modest data review shows that we know a considerable amount about the relatively short-term consequences of various situations that occur before and at the time of conception. Studies have shown that numerous xenobiotics are present in semen and follicular fluid, or circulating in maternal blood and lodged in fat stores. The expanding ART literature clearly documents that the environment at the time of conception can impact the developing embryo, and thus likely affects the long-term health of the child, and thus affects healthcare spending and its associated burdens. Nutrition clearly impacts the progress of the pregnancy and the subsequent health of the child in many known ways.

This review documents what is already known about the determinants of children's health. Knowing that we only make new findings (i.e., become smarter) when we ask new questions, the implication that cannot be ignored is that the long-term longitudinal National Children's Study (2002) is our best hope for actually testing some of the potential effects identified in this literature, and for identifying new associations between early exposures and later health status. Only by collecting biological specimens and actually measuring what the internal exposures are in women and men prior to and at the time of conception will we be able to definitively say which, if any, of these exposures are meaningful. Doing this in the context of a large longitudinal epidemiologic health study will help us determine which of these exposures impacts the health of the offspring. Knowing this can powerfully inform future decision-making, will create hypotheses to test and verify for years to come, and will allow regulatory efforts to focus on those exposures that have the greatest impact. This is a chance that must not be missed.

References

Alikani M, Noyes N, Cohen J, Rosenwaks Z. 1994. Monozygotic twinning in the human is associated with the zona pellucida architecture. Hum Reprod 9:1318–1321.

Allen LH. 2001. Biological mechanisms that might underlie iron's effects on fetal growth and preterm birth. J Nutr 131:581S–589S.

Allen WR, Pashen RL. 1984. Production of monozygotic (identical) horse twins by embryo micromanipulation. J Reprod Fertil 71:607–613.

Anderson D, Dobrzynska MM, Basaran N. 1997. Effect of various genotoxins and reproductive toxins in human lymphocytes and sperm in the comet assay. Teratog Carcinog Mutagen 17:29–43.

Arbuckle TE, Schrader SM, Cole D, Hall JC, Bancej CM, Turner LA, et al. 1999. 2,4-Dichloropheoxyacetic acid residues in semen of Ontario farmers. Reprod Toxicol 13:421–429.

Ashworth CJ, Antipatis C. 2001. Micronutrient programming of development throughout gestation. Reproduction 122:527–535.

ATSDR. 2000. Toxicological Profile for Polychlorinated Biphenyls (PCBs). Atlanta, GA:Agency for Toxic Substances and Disease Registry.

Balen AH, Tan S-L, MacDougall J, Jacobs HS. 1993. Miscarriage rates following *in-vitro* fertilization are

increased in women with polycystic ovaries and reduced by pituitary desensitization with buserilin. Hum Reprod 8:959–964.

Barlow S, Kavlock RJ, Moore JA, Schantz SL, Sheehan DM, Shuey DL, et al. 1999. Teratology Society Public Affairs Committee position paper: developmental toxicity of endocrine disruptors to humans. Teratology 60:365–375.

Bavistor BD. 1999. Glucose and culture of human embryos. Fertil Steril 72:233–234.

Behr B, Fisch JD, Racowsky C, Miller K, Pool TB, Milki AA. 2000. Blastocyst-ET and monozygotic twinning. J Assist Reprod Genet 17:349–351.

Bergh T, Ericson A, Hillensjo T, Nygren KG, Wennerholm UB. 1999. Deliveries and children born after *in-vitro* fertilization in Sweden 1982–95: a retrospective cohort study. Lancet 354:1579–1585.

Blair A, Tarone R, Sandler D, Lynch CF, Rowland A, Wintersteen W, et al. 2001. Reliability on reporting on lifestyle and agricultural factors by a sample of participants in the agricultural health study from Iowa. Epidemiology 13:94–99.

Blanco J, Gabau E, Gomez D, Baena N, Guitart M, Egozcue J, et al. 1998. Chromosome 21 disomy in the spermatozoa of fathers of children with trisomy 21 in a population with a high prevalence of Down syndrome: increased incidence in cases of paternal origin. Am J Hum Genet 63:1067–1072.

Blount BC, Silva MJ, Caudill SP, Needham LL, Pirkle JL, Sampson EJ, et al. 2000. Levels of seven urinary phthalate metabolites in a human reference population. Environ Health Perspect 108(10):979–982.

Bonduelle M, Liebaers I, Deketelaere V, Derde MP, Camus M, Devroey P, et al. 2002. Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999). Hum Reprod 17:671–694.

Botto LD, Khoury MJ, Mulinare J, Erickson JD. 1996. Periconceptional multivitamin use and the occurrence of conotruncal heart defects: results from a population-based, case-control study. Pediatrics 98:911–917.

Caan B, Horgen DM, Margen S, King JC, Jewell NP. 1987. Benefits associated with WIC supplemental feeding during the interpregnancy interval. Am J Clin Nutr 45:29–41.

Center for the Evaluation of Risks to Human Reproduction (CERHR). 2002. Summary of Phthalate-Related Reports. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://cerhr.niehs.nih.gov [accessed 10 Feb 2003].

CDC. 2000. Blood lead levels in young children—United States and selected states, 1996–1999. MMWR Morb Mortal Wkly Rep 49:1133–1337.

———. American Society for Reproductive Medicine, Society for Assisted Reproductive Technology, RESOLVE. 2002a. Assisted reproductive technology success rates: national summary and fertility clinic reports. Atlanta, GA:Centers for Disease Control and Prevention.

———. 2002b. Use of assisted reproductive technology – United States, 1996 and 1998. MMWR Morb Mortal Wkly Rep 51:97–101.

Chung EK, Webb D, Clampet-Lundquist S, Campbell C. 2001. A comparison of elevated blood lead levels among children living in foster care, their siblings, and the general population [Abstract]. Pediatrics 107:E81.

Cnattingius S, Bergstrom R, Lipworth L, Kramer M. 1998. Prepregnancy weight and the risk of adverse pregnancy outcomes. N Engl J Med 338(3):147–152.

Coates A, Rutherford AJ, Hunter H, Leese HJ. 1999. Glucose-free medium in human *in vitro* fertilization and embryo transfer: a large-scale, prospective, randomized clinical trial. Fertil Steril 72:229–232.

Colon I, Caro D, Bourdony CJ, Rosario O. 2000. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environ Health Perspect 108:895–900.

Conaghan J, Hardy K, Leese HJ, Winston RM, Handyside AH. 1998. Culture of human preimplantation embryos to the blastocyst stage: a comparison of 3 media. Int J Dev Biol 42:885–893.

Cummings AM. 1993. Assessment of implantation in the rat. In: Methods in Toxicology (Chapin RE, Heindel JJ, eds). Vol 3(B). San Diego; Academic Press, 194–198.

Czeizel AE. 1995. Nutritional supplementation and prevention of congenital abnormalities. Curr Opin Obstet Gynecol 7:88–94.

———. 1996. Reduction of urinary tract and cardiovascular defects by periconceptional multivitamin supplementation. Am J Med Genet 62:179–183.

da Costa ALE, Abdelmassih SA, de Oliverira FG, Abdelmassih R, Nagy ZP, Balmaceda JP, et al. 2001. Monozygotic twins and transfer at the blastocyst stage after ICSI. Hum Reprod 16:333–336.

Davis DL, Gottlieb MB, Stampnitzky JR. 1998. Reduced rate of male to female births in several industrial countries: a sentinel health indicator? JAMA 279:1018–1023.

Dawson EB, Evans DR, Harris WA, Powell LC. 2000. Seminal plasma trace metal levels in industrial workers. Biol Trace Elem Res 74:97–105.

Derom C, Derom R, Vlietinck R, Van den Berghe H, Thiery M. 1987. Increased monozygotic twinning rate after ovulation induction. Lancet 1:1236–1238.

Desai M, Crowther NJ, Lucas A, Hales CN. 1996. Organ-selective growth in the offspring of protein-restricted mothers. Br J Nutr 76:591–603.

Devreker F, Hardy K, Van den Bergh M, Vannin AS, Emiliani S, Englert Y. 2001. Amino acids promote human blastocyst development *in vitro*. Hum Reprod 16(4):749–756.

Dhont M, De Sutter P, Ruyssink G, Martens G, Bekaert A. 1999. Perinatal outcome of pregnancies after assisted reproduction: a case-control study. Am J Obstet Gynecol 181:688–695.

Dickey RP, Taylor SN, Curolw DN, Rye PH, Pyrzak R. 1996. Incidence of spontaneous abortion in clomiphene pregnancies. Hum Reprod 11:2623–2628.

Dolk H. 1998. Rise in prevalence of hypospadias [Letter]. Lancet 351(9105):770.

Doyle P, Maconochie N, Roman E, Davies G, Smith PG, Beral V. 2000. Fetal death and congenital malformation in babies born to nuclear industry employees: report from the nuclear industry family study. Lancet 356:1293–1299.

Dunn A, Macfarlane A. 1996. Recent trends in the incidence of multiple births and associated mortality in England and Wales. Arch Dis Child Fetal Neonatal Ed 75:F10–F19.

Ericson A, Kallen B. 2001. Congenital malformations in infants born after IVF: a population-based study. Hum Reprod 16:504–509.

Eskenazi B, Wyrobek AJ, Kidd SA, Lowe X, Moore D II, Weisiger K, et al. 2002. Sperm aneuploidy in fathers of children with paternally and maternally inherited Klinefelter syndrome. Hum Reprod 17:576–583.

Evenson DP. 1999. Alterations and damage of sperm chromatin structure and early embryonic failure. In: Towards Reproductive Certainty: Fertility and Genetics Beyond 1999 (Jannsen R, Mortimer D, eds). New York:Parthenon Publishing Group Ltd, 313–329.

Evenson DP, Larsen KL, Jost LK. 2002. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 23:25–43.

Farrow A, Farrow SC, Little R, Golding J, The Alspac Study Team. 1996. The repeatability of self-reported exposure after miscarriage. Int J Epidemiol 25(4):797–806.

Fein G, Jacobson JL, Jacobson SW, Schwartz PM, Dowler JK. 1984. Prenatal exposure to polychlorinated biphenyls: effects on birth size and gestational age. J Pediatr 105:310–315.

Feldman Y, Koren G, Mattice D, Shear H, Pellegrini E, MacLeod S. 1989. Determinants of recall and recall bias in studying drug and chemical exposure in pregnancy. Teratology 40:37–45.

FIVNAT (French *in Vitro* National). 1995. Pregnancies and births resulting from *in vitro* fertilization: French National Registry, analysis of data 1986 to 1990. Fertil Steril 64:746–756.

Fraga CG, Motchnik PA, Wyrobek AJ, Rempel DM, Ames BN. 1996. Smoking and low antioxidant levels increase oxidative damage to sperm DNA. Mutat Res 351:199–203.

Friedler S, Mashiach S, Laufer N. 1992. Births in Israel resulting from *in-vitro* fertilization/embryo transfer, 1982–1989: National Registry of the Israeli Association for Fertility Research. Hum Reprod 7:1159–1163.

Fukuda M, Fukuda K, Shimizu T, Andersen CY, Byskov AG. 2002. Parental periconceptional smoking and male:female ratio of newborn infants. Lancet 359:1407–1408.

Gardner DK, Pool TB, Lane M. 2000. Embryo nutrition and energy metabolism and its relationship to embryo growth, differentiation, and viability. Semin Reprod Med 18:205–218.

Geist R, Koren G. 2001. Maternal disorders leading to increased reproductive risks. In: Maternal-Fetal Toxicology, A Clinician's Guide (Koren G, ed). New York:Marcel Dekker, 697–732.

Generoso WM, Katoh M, Cain KT, Hughes LA, Foxworth LB, Mitchell TJ, et al. 1989. Chromosome malsegregation and embryonic lethality induced by treatment of normally ovulated mouse oocytes with nocodazole. Mutat Res 210:313–322.

Generoso WM, Rutledge JC, Cain KT, Hughes LA, Braden PW. 1987. Exposure of female mice to ethylene oxide within hours after mating leads to fetal malformation and death. Mutat Res 176:269–274.

Gillman MW. 2002. Epidemiological challenges in studying the fetal origins of adult chronic disease. Int J Epidemiol 31:294–299.

Gissler M, Silvero MM, Hemminki E. 1995. *In-vitro* fertilization pregnancies and perinatal health in Finland 1991–1993. Hum Reprod 10:1856–1861.

Gonzales CH, Marques-Dias MJ, Kim CA, Sugayama SM, Da Paz JA, Huson SM, et al. 1998. Congenital abnormalities in Brazilian children associated with misoprostol misuse in first trimester of pregnancy. Lancet 351:1624–1627.

Gorrill MJ, Sadler-Fredd KS, Patton PE, Burry KA. 2001. Multiple gestations in assisted reproductive technology: can they be avoided with blastocyst transfers? Am J Obstet Gynecol 184:1471–1477.

Grandjean P, Weihe P, White RF, Debes F. 1998. Cognitive performance of children prenatally exposed to "safe" levels of methylmercury. Environ Res 77:165–172.

Gray LE. 1998. Xenoendocrine disrupters: laboratory studies on male reproductive effects. Toxicol Lett 102:331–335.

Gressens P, Muaku SM, Besse L, Nsegbe E, Gallego J, Delpech B, et al. 1997. Maternal protein restriction early in rat pregnancy alters brain development in the progeny. Brain Res Dev Brain Res 103(1):21–35.

Guttmacher AF. 1953. The incidence of multiple births in man and some of the other unipara. Obstet Gynecol 2:22–35.

Guvenc H, Karatas F, Guvenc M, Kunc S, Aygun AD, Bektas S. 1995. Low levels of selenium in mothers and their newborns in pregnancies with a neural tube defect. Pediatrics 95:879–882.

Hales BF, Robaire B. 2001. Paternal exposure to drugs and environmental chemicals: effects on progeny outcome. J Androl 22:927–936.

Hall J, Solehdin F. 1998. Folic acid for the prevention of congenital anomalies. Eur J Pediatr 157:445–450.

Hama Y, Uematsu M, Sakurai Y, Kusano S. 2001. Sex ratio in the offspring of male radiologists. Acad Radiol 8:421–424.

Hammadeh ME, Al-Hassani S, Stieber M, Rosenbaum P, Kupker D, Diedrish K. 1996. The effect of chromatin condensation (aniline blue staining) and morphology (strict criteria) of human spermatozoa on fertilization, cleavage, and pregnancy rates in an intracytoplasmic sperm injection programme. Hum Reprod 11:2468–2471.

Hanrahan LP, Falk C, Anderson HA, Draheim L, Kanarek MS, Olson J, and The Great Lakes Consortium. 1999. Serum PCB and DDE levels of frequent Great Lakes sport fish consumers—a first look. Environ Res 80:S26–S37.

Hansen M, Kurinczuk JJ, Bower C, Webb S. 2002. The risk of major birth defects after intracytoplasmic sperm injection and *in vitro* fertilization. N Engl J Med 346:725–730.

Harkonen K, Viitanen T, Larsen SB, Bonde JP, ASCLEPIOS, Lahdetie J. 1999. Aneuploidy in sperm and exposure to fungicides and lifestyle factors. Environ Mol Mutagen 34:39–46.

Harlow SD, Linet MS. 1989. Reviews and commentary agreement between questionnaire data and medical records. The evidence for accuracy of recall. Am J Epidemiol 129(2):233–248.

Hassold TJ. 1998. Nondisjunction in the human male. In: Meiosis and Gametogenesis. San Diego, CA:Academic Press, 383–406.

Hershlag A, Paine T, Cooper GW, Scholl GM, Rawlinson K, Kvapil G. 1999. Monozygotic twinning associated with mechanical assisted hatching. Fertil Steril 71:144–146.

Hewitson L, Dominko T, Takahashi D, Martinovich C, Ramalho-Santos J, Sutovsky P, et al. 1999. Unique checkpoints during the first cell cycle of fertilization after intracytoplasmic sperm injection in rhesus monkeys. Nature Med 5:431–433.

Hixon M, Millie E, Judis LA, Sherman S, Allran K, Taft L. 1998. FISH studies of sperm of fathers of paternally derived cases of trisomy 21: no evidence for an increase in aneuploidy. Hum Genetics 103:654–657.

Hoffman D, Brunnemann KD, Prokopczyk B, Djordjevic MV. 1994. Tobacco-specific *N*-nitrosamines and areca-derived *N*-nitrosamines—chemistry, biochemistry, carcinogenicity, and relevance to humans. J Toxicol Environ Health 41:1–52.

Humphrey HEB, Gardiner JC, Pandya JR, Sweeney AM, Gasior DM, McCaffrey RJ, et al. 2001. PCB congener profile in the serum of humans consuming Great Lakes fish. Environ Health Perspect 108:167–172.

Hur YM, McGue M, Iacono WG. 1995. Unequal rate of monozygotic and like-sex dizygotic twin birth: evidence from the Minnesota twin family study. Behav Genet 25:337–340.

Iannaccone PM, Bossert NL, Connelly CS. 1987. Disruption of embryonic and fetal development due to preimplantation chemical insults: a critical review. Am J Obstet Gynecol 157:476–484.

Inskip H. 1999. Stillbirth and paternal preconceptional radiation exposure. Lancet 354:1400–1401.

Itikala PR, Watkins ML, Mulinare J, Moore CA, Liu Y. 2001. Maternal multivitamin use and orofacial clefts in offspring. Teratology 63:79–86.

Jacobson JL, Jacobson SW. 1996. Intellectual impairment in children exposed to polychlorinated biphenyls *in utero*. N Engl J Med 335:783–789.

James WH. 1996. Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels at the time of conception. J Theor Biol 180:271–286.

Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. 1995. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect 106:582–587.

Kallen B, Bertollini R, Castilla E, Gzeizel A, Knudsen LB, Martinas-Frias ML. 1986. A joint international study of the epidemiology of hypospadias. Acta Paediatr Scand 324(suppl):1–52.

Kapoor JP, Metcalf RL, Nystrom RF, Sangha GK. 1970. Comparative metabolism of methoxychlor, methiochlor, and DDT in mouse, insects, and in a model ecosystem. J Agric Food Chem 18:1145–1152.

Kelly JP, Rosenberg KL, Kaufman DW, Shapiro S. 1990. Reliability of personal interview data in a hospital-based case-control study. Am J Epidemiol 131(1):79–90.

Khosla S, Dean W, Reik W, Feil R. 2001. Epigenetic and experimental modifications in early mammalian development: Part II. Culture of preimplantation embryos and its long-term effects on gene expression and phenotype. Hum Reprod Update 7:419–427.

King JC. 2000. Physiology of pregnancy and nutrient metabolism. Am J Clin Nutr 71(suppl):1218S–1225S.

Kirke PN, Molloy AM, Daly LE, Burke H, Weir DG, Scott JM. 1993. Maternal plasma folate and vitamin B12 are independent risk factors for neural tube defects. Q J Med 86:703–708.

Klip H, Verloop J, van Gool JD, Koster ME, Burger CW, van Leeuwen FE. 2002. Hypospadias in sons of women exposed to diethylstilbestrol *in utero*: a cohort study. Lancet 359:1102–1107.

Kovacs BW, Kirschbaum TH, Paul RH. 1989. Twin gestations. I. Antenatal care and complications. Obstet Gynecol 74:313–317.

Kreiss K. 1985 Studies on populations exposed to polychlorinated biphenyls. Environ Health Perspect 60:193–199.

Kumar R, Pant N, Srivastava SP. 2000. Chlorinated pesticides and heavy metals in human semen. Int J Androl 23:145–149.

Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP. 2000. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. Development 127:4195–4202.

Laden F, Collman G, Iwamoto K, Alberg AJ, Berkowitz GS, Freudenheim JL, et al. 2001. 1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene and polychlorinated biphenyls and breast cancer: combined analysis of five U.S. studies. J Natl Cancer Inst 93:768–775.

Langley-Evans SC. 2000. Critical differences between two low protein diet protocols in the programming of hypertension in the rat. Int J Food Sci Nutr 51:11–17.

Lelong N, Kaminski M, Saurel-Cubizolles MJ, Bouvier-Colle MH. 2001. Postpartum return to smoking among usual smokers who quit during pregnancy. Eur J Public Health 11(3):334–339.

London SN, Young DM, Caldito G, Maihes JB. 2000. Clomiphene citrate-induced perturbations during meiotic maturation and cytogenetic abnormalities in mouse oocytes *in vivo* and *in vitro*. Fertil Steril 73:620–626.

Lu ZM, Goldenberg RL, Cliver SP, Cutter G, Blankson ML. 1991. The relationship between maternal hematocrit and pregnancy outcome. Obstet Gynecol 71:190–194.

Luetjens CM, Payne C, Schatten G. 1999. Non-random chromosome positioning in human sperm and sex chromosome anomalies following intracytoplasmic sperm injection. Lancet 353:1240.

Luke B. 1994a. Nutrition during pregnancy. Curr Opin Obstet Gynecol 6:402–407.

———. 1994b. Nutritional influences on fetal growth. Clin Obstet Gynecol 37:538–549.

Luke MC, Coffey DS. 1994. The male accessory tissues: structure, androgen action, and physiology. In: The Physiology of Reproduction (Knobil E, Neill JD, eds). Second ed, Vol 1. New York:Raven Press, 1478.

Lumley J, Watson L, Watson M, Bower C. 2002. Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects (Cochrane Review). In: The Cochrane Library. Issue 2. Oxford, UK:Update Software.

MacGillivray I. 1986. Epidemiology of twin pregnancy. Semin Perinatol 10:4–8.

MacIntosh DL, Needham LL, Hammerstrom KA, Ryan PB. 1999. A longitudinal investigation of selected pesticide metabolites in urine. J Expo Anal Environ Epidemiol 9:949–501.

Martin JA, Park MM. 1999. Trends in twin and triplet births: 1980–97. National Vital Statistics Reports. Vol 47, no 24. Hyattsville, MD:National Center for Health Statistics.

Martinez-Pasarell O, Nogues C, Bosch M, Egozcve J, Templado C. 1999. Analysis of sex chromosome aneuploidy in sperm from fathers of Turner syndrome patients. Hum Genet 3:1365–1371.

McElhatton PR, Bateman DN, Pughe KR, Thomas SH. 1999. Congenital anomalies after prenatal ecstasy exposure. Lancet 354:1441–1442.

McFaul PB, Patel N, Mills J. 1993. An audit of the obstetric outcome of 148 consecutive pregnancies from assisted conception: implications for neonatal services. Br J Obstet Gynaecol 100:820–825.

Metges CC. 2001. Does dietary protein in early life effect the development of adiposity in mammals? J Nutr 131:2062–2066.

Mocarelli P, Gerthoux PM, Ferrari E, Patterson DG Jr, Kicszak SM, Brambilla P. 2000. Paternal concentrations of dioxin and sex ratio of offspring. Lancet 355:1858–1863.

MRC Working Party on Children Conceived by *in Vitro* Fertilisation. 1990. Births in Great Britain resulting from assisted conception, 1978-87. Br Med J 300:1229–1233.

Murphy R, Harvey C. 1985. Residues and metabolites of selected persistent halogenated hydrocarbons in blood specimens from a general population survey. Environ Health Perspect 60:115–120.

Naeye RL, Tafari N, Judge D, Marboe CC. 1978. Twins: causes of perinatal death in 12 United States cities and one African city. Am J Obstet Gynecol 131:267–272.

National Children's Study. 2002. Home Page. Bethesda, MD:National Institutes of Health. Available: http://nationalchildrensstudy.gov [accessed 9 Feb 2003].

National Research Council. 2000. Toxicological Effects of Mercury. Washington, DC:Committee on the Toxicological Effects of Methylmercury, National Research Council.

Nijs M, Vanderzwalmen P, Segal-Bertin G, Geerts L, Van Roosendaal E, Segal L, et al. 1993. A monozygotic twin pregnancy after application of zona rubbing on a frozen-thawed blastocyst. Hum Reprod 8:127–129.

Noack-Fuller G, De Beer C, Seibert H. 1993. Cadmium, lead, selenium, and zinc in semen of occupationally unexposed men. Andrologia 25:7–12.

O'Rahilly R, Muller F. 2001. Human Embryology & Teratology. 3rd ed. New York:Wiley-Liss.

Oktay K, Berkowitz P, Berkus M, Schenken RS, Brzyski RG. 2000. The re-incarnation of an old question—clomid effect on oocyte and embryo? Fertil Steril 74:422–423.

Olivennes F, Rufat P, Andre B, Poourade A, Quiros MC, Frydman R. 1993. The increased risk of complication observed in singleton pregnancies resulting from *in-vitro* fertilization (IVF) does not seem to be related to the IVF method itself. Hum Reprod 8:1297–1300.

Pacifici R, Altieri I, Gandini L, Lenzi A, Pichini S, Rosa M, et al. 1993. Nicotine, cotinine, and *trans*-3-hydroxycotinine levels in seminal plasma of smokers: effects on sperm parameters. Ther Drug Monit 15:358–363.

Padungtod C, Hassold T, Millie E, Ryan LM, Savitz DA, Christian DC, et al. 1999. Sperm aneuploidy among Chinese pesticide factory workers: scoring by the FISH method. Am J Ind Med 36:230–238.

Pangalos C, Talbot C, Lewis J, Adelsberger P, Petersen M, Serre J-L, et al. 1992. DNA polymorphism analysis in families with recurrence of free trisomy 21. Am J Hum Genet 51:1015–1027.

Parazzini F, Tozzi L, Mezzanotte G, Bocciolone L, Vecchia CL, Fedele L, et al. 1991. Trends in multiple births in Italy: 1955–1983. Br J Obstet Gynaecol 98:535–539.

Paulozzi LJ, Erickson JD, Jackson RJ. 1997. Hypospadias trends in two US surveillance systems. Pediatrics 100:831–834.

Peramo B, Ricciarelli E, Cuadros-Fernandez JM, Huguet E, Hernandez ER. 1999. Blastocyst transfer and monozygotic twinning. Fertil Steril 72:1116–1117.

Perreault SD, Aitken RJ, Baker HWG, Evenson DP, Huszar G, Irvine DS, et al. 2003. Integrating new tests of sperm genetic integrity into semen analysis: breakout group discussion. In: Advances in Male Mediated Developmental Toxicity (Robaire B, Hales BF, eds). New York:Kluwer Academic/Plenum, 253–268.

Perreault SD, Rubes J, Robbins WA, Evenson DP, Selevan SG. 2000. Evaluation of aneuploidy and DNA damage in human spermatozoa: applications in field studies. Andrologia 32:247–254.

Perry GS, Byers TE, Mokdah AH, Serdula MK, Williamson DF. 1995. The validity of self-reports of past body weight by U.S. adults. Epidemiology 6:61–66.

Pflieger-Bruss S, Schill W-B. 2000. Effects of chlorinated hydrocarbons on sperm function *in vitro*. Andrologia 32:311–315.

Picard L, King WA, Betteridge KJ. 1985. Production of sexed calves from frozen-thawed embryos. Vet Rec 117:603–608.

Potashnik G, Goldsmith J, Insler V. 1984. Dibromochloropropane-induced reduction of the sex ratio in man. Andrologia 16(3):213–218.

Potts RJ, Newbury CJ, Smith G, Notarianni LJ, Jefferies TM. 1999. Sperm chromatin damage associated with male smoking. Mutat Res 423:103–111.

Pryor JL, Hughes C, Foster W, Hales BF, Robaire B. 2000. Critical windows of exposure for children's health: the reproductive system in animals and humans. Environ Health Perspect 108(suppl 3):491–503.

Ramakrishnan U, Manjrekar R, Rivera J, Gonzales-Cossio T, Martorell R. 1999. Micronutrients and pregnancy outcome: a review of the literature. Nutr Res 19(1):103–159.

Ramalho-Santos J, Sutovshy P, Simerly C, Oko R, Wessel GM, Hewitson L, et al. 2000. ICSI choreography: fate of sperm structures after monospermic rhesus ICSI and first cell cycle implications. Hum Reprod 15:2610–2620.

Rasmussen KM. 2001. Is there a causal relationship between iron deficiency or iron-deficiency anemia and weight at birth, length of gestation and perinatal mortality? J Nutr 131:590S–603S.

Recio R, Robbins WA, Borja-Aburto V, Moran-Martinez J, Froines JR, Hernandez RM, et al. 2001. Organophosphorous pesticide exposure increases the frequency of sperm sex null aneuploidy. Environ Health Perspect 109(12):1237–1240.

Rivrud GN. 1988. Mutagenicity testing of seminal fluid: seminal fluid increases the mutagenicity of the precursor mutagen benzo[*a*]pyrene in the presence of S9 mix. Mutat Res 208:195–200.

Robbins WA, Rubes J, Selevan SG, Perreault SD. 1999. Air pollution and sperm aneuploidy in healthy young men. Environ Epidemiol Toxicol 1:125–131.

Robbins WA, Vine M, Truong K, Everson RB. 1997. Use of fluorescence *in situ* hybridization (FISH) to assess effects of smoking, caffeine, and alcohol on aneuploidy load in sperm of healthy men. Environ Mol Mutagen 30:175–183.

Robinson J, Chidzanja S, Kind K, Lok F, Owens P, Owens J. 1995. Placental control of fetal growth. Reprod Fertil Dev 7:333–344.

Robinson JJ, Sinclair KD, McEvoy TG. 1999. Nutritional effects of foetal growth. Anim Sci 68:315–331.

Rogan WJ, Gladen BG, Guo Y-L L, Hsu C-C. 1999. Sex ratio after exposure to dioxin-like chemicals in Taiwan. Lancet 353:206–207.

Root R, Browner CH. 2001. Practices of the pregnant self: compliance with and resistance to prenatal norms. Cult Med Psychiatry 25(2):195–223.

Rothman KJ, Moore LL, Singer MR, Nguyen US, Mannino S, Milunsky A. 1995. Teratogenicity of high vitamin A intake. N Engl J Med 23:1369–1373.

Rubes J, Lowe X, Moore HD, Perreault S, Slott V, Evenson D, et al. 1998. Smoking cigarettes is associated with increased sperm disomy in teenage men. Fertil Steril 70:715–723.

Rutledge JC, Generoso WM, Shourbaji A, Cain KT, Gans M, Oliva J. 1992. Developmental anomalies derived from exposure of zygotes and first-cleavage embryos to mutagens. Mutat Res 296:167–177.

Saaranen M, Kantola M, Saarikoski S, Vanha-Perttula T. 1989. Human seminal plasma cadmium: comparison with fertility and smoking habits. Andrologia 21:140–145.

Sadler TW. 1998. Mechanisms of neural tube closure and defects. Ment Retard Dev Disabil Res Rev 4:247–253.

Sakamoto M, Nakano A, Akagi H. 2001. Declining Minamata male birth ratio associated with increased male fetal death due to heavy methylmercury pollution. Environ Res 87:92–97.

Sakkas D, Urner F, Bizzaro D Manicardi G, Bianchi PG, Shoukir Y. 1998. Sperm nuclear DNA damage and altered chromatin structure: effect on fertilization and embryo development. Hum Reprod 4(suppl):11–19.

Sanborn MD, Abelsohn A, Campbell M, Weir E. 2002. Identifying and managing adverse environmental health effects. 3. Lead exposure. Can Med Assoc J 166:1287–1292.

Scanlon KS, Yip R, Schieve LA, Cogswell ME. 2000. High and low hemoglobin levels during pregnancy: differential risks for preterm birth and small for gestational age. Obstet Gynecol 96(5 pt 1):741–748.

Schantz SL, Gasior DM, Ploverejan E, McCaffrey RJ, Sweeney AM, Humphrey HE, et al. 2001. Impairments of memory and learning in older adults exposed to polychlorinated biphenyls via consumption of Great Lakes fish. Environ Health Perspect 109:605–611.

Schecter A, McGee H, Stanley JS, Boggess K, Brandt-Rauf P. 1996. Dioxins and dioxin-like chemicals in blood and semen of American Vietnam veterans from the state of Michigan. Am J Ind Med 30:647–654.

Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. 2002. Low and very low birth weight in infants conceived with use of assisted reproductive technology. N Engl J Med 346:731–737.

Schieve LA, Meikle SF, Peterson HB, Jeng G, Danel I, Burnett NM, et al. 2000. Does assisted hatching pose a risk for monozygotic twinning in pregnancies conceived through *in vitro* fertilization? Fertil Steril 74:288–294.

Schlebusch H, Wagner U, van der Ven H, Al-Hasani S, Diedrich K, Krebs D. 1989. Polychlorinated biphenyls: the occurrence of the main congeners in follicular and sperm fluids. J Clin Chem Clin Biochem 27:663–667.

Scholl TO, Hediger ML, Fischer RL, Shearer JW. 1992. Anemia versus iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr 55:985–988.

Scholl TO, Reilly T. 2000. Anemia, iron and pregnancy outcome. J Nutr 130:443S–447S.

Seidel SD, Li V, Winter GM, Rogers WJ, Martinez EI, Denison MS. 2000. Ah receptor-based chemical screening bioassays: application and limitations for the detection of Ah receptor agonists. Toxicol Sci 55:107–115.

Selevan SG, Kimmel CA, Mendola P. 2000. Identifying critical windows of exposure for children's health. Environ Health Perspect 108(suppl 3):451–455.

Sempos CT. 1992. Invited commentary: Some limitations of semi-quantitative food frequency questionnaires. Am J Epidemiol 135:1127–1132.

Sergerie M, Ouhilal S, Bissonnette F, Brodeur J, Bleau G. 2000. Lack of association between smoking and DNA fragmentation in the spermatozoa of normal men. Hum Reprod 15:1314–1321.

Sever LE, Gilbert ES, Hessol NA, McIntyre JM. 1988. A case-control study of congenital malformations and occupational exposure to low-level ionizing radiation. Am J Epidemiol 127:226–242

Shadel BN, Evans RG, Roberts D, Clardy S, Jordan-Izzaguire D, Patterson DG Jr, et al. 2001. Background levels of non-*ortho*-substituted (coplanar) polychlorinated biphenyls in human serum of Missouri residents. Chemosphere 43(4–7):967–976.

Sheiner E, Har-Vardi I, Potashinik G. 2001. The potential association between blastocyst transfer and monozygotic twinning. Fertil Steril 75:217–218.

Shiota K, Chou MJ, Nishimura H. 1980. Embryotoxic effects of di-2-ethylhexyl phthalate (DEHP) and di-*n*-butyl phthalate (DBP) in mice. Environ Res 22:245–253.

Shoham Z, Zosmer A, Insler V. 1991. Early miscarriage and fetal malformations after induction of ovulation (by clomiphene citrate and/or human menotropins), *in vitro* fertilization, and gamete intrafallopian transfer. Fertil Steril 55:1–11.

Shoob HD, Sargent RG, Thompson SJ, Best RG, Drane JW, Tocharoen A. 2001. Dietary methionine is involved in the etiology of neural tube defect-affected pregnancies in humans. J Nutr 131:2653–2658.

Skupski DW, Streltzoff J, Hutson M, Rosenwaks Z, Cohen J, Chervenak FA. 1995. Early diagnosis of conjoined twins in triplet pregnancy after *in vitro* fertilization and assisted hatching. J Ultrasound Med 14:611–615.

Slotnick RN, Ortega JE. 1996. Monoamniotic twinning and zona manipulation: a survey of U.S. IVF centers correlating zona manipulation procedures and high-risk twinning frequency. J Assist Reprod Genet 13:381–385.

Sorensen HT, Thulstrup AM, Mortensen J, Larsen H, Pedersen L. 2000. Hyperemesis gravidarum and sex of child. Lancet 355(9201):407.

Sotomaru Y, Kato Y, Tsunoda Y. 1998. Production of monozygotic twins after freezing and thawing of bisected mouse embryos. Cryobiology 37:139–145.

Stachel B, Dougherty RC, Dahl U, Schlosser M, Zeschmar B. 1989. Toxic environmental chemicals in human semen: analytical method and case studies. Andrologia 21:282–291.

Stevens-Simon C, McAnarney ER, Coulter MP. 1986. How accurately do pregnant adolescents estimate their weight prior to pregnancy? J Adolesc Health Care 7:250–254.

Stromberg B, Dahlquist G, Ericson A, Finnstrom O, Koster M, Stjernqvist KK. 2002. Neurological sequelae in children born after *in-vitro* fertilisation: a population-based study. Lancet 359:461–465.

Sundstrom I, Ildgruben A, Hogberg U. 1997. Treatment-related and treatment-independent deliveries among infertile couples, a long-term follow-up. Acta Obstet Gynecol Scand 76:238–243.

Susser M, Stein Z. 1994. Timing in prenatal nutrition: a reprise of the Dutch Famine Study. Nutr Rev 52(3):84–94.

Sutovsky P, Hewitson L, Simerly CR, Tengowski MW, Navara CS, Haavisto A, et al. 1996. Intracytoplasmic sperm injection for rhesus monkey fertilization results in unusual chromatin, cytoskeletal, and membrane events, but eventually leads to pronuclear development and sperm aster assembly. Hum Reprod 11:1703–1712.

Szymczynski GA, Waliszewski SM. 1981. Content of chlorinated pesticides in human semen of a random population. Int J Androl 4:669–674.

Talansky BE, Gordon JW. 1988. Cleavage characteristics of mouse embryos inseminated and cultured after zona pellucida drilling. Gamete Res 21:277–287.

Tanaka A, Matsumoto A, Yamaha T. 1978. Biochemical studies on phthalic esters. III. Metabolism of dibutyl phthalate (DBP) in animals. Toxicology 9:109–123.

Tee PG, Sweeney AM, Symanski E, Gardiner JC, Gasior DM, Schantz SL. 2003. A longitudinal examination of factors related to changes in serum polychlorinated biphenyl levels. Environ Health Perspect 111(5):702–707.

Telisman S, Cvitkovic P, Jurasovic J, Piaent A, Gavella M, Rocic B. 2000. Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. Environ Health Perspect 108:45–53.

Terada Y, Luetjens CM, Sutovshy P, Schatten G. 2000. Atypical decondensation of the sperm nucleus, delayed replication of the male genome, and sex chromosome positioning following intracytoplasmic sperm injection (ICSI) into golden hamster eggs: does ICSI itself introduce chromosomal anomalies? Fertil Steril 74:454–456.

Tesarik J, Mendoza C, Greco E. 2002. Paternal effects acting during the first cell cycle of human preimplantation development after ICSI. Hum Reprod 17:184–189.

Toppari J, Larsen J, Christiansen P, Giwercman A, Grandjean P, Guillette LJ, et al. 1996. Male reproductive health and environmental xenoestrogens. Environ Health Perspect 104(suppl 4):741–803.

Van Langendonckt A, Wyns C, Godin PA, Toussaint-Demylle D, Donnez J. 2000. Atypical hatching of a human blastocyst leading to monozygotic twinning: a case report. Fertil Steril 74:1047–1050.

Velie EM, Block G, Shaw GM, Samuels SJ, Schaffer DM, Kulldoff M. 1999. Maternal supplementation and dietary zinc intake and the occurrence of neural tube defects in California. Am J Epidemiol 150:605–616.

Verlaenen H, Cammu H, Derde MP, Amy JJ. 1995. Singleton pregnancy after *in vitro* fertilization expectations and outcome. Obstet Gynecol 86:906–910.

Wagner U, Schlebusch H, van der Ven H, Diedrich K, Krebs D. 1990. Accumulation of pollutants in the genital tract of sterility patients. J Clin Chem Clin Biochem 28:683–688.

Wei EK, Gardner J, Field AE, Rosner BA, Colditz GA, Suitor CW. 1999. Validity of a food frequency questionnaire in assessing nutrient intakes of low-income pregnant women. Matern Child Health J 3(4):241–246.

Wells JC, Cole TJ. 2002. Birth weight and environmental heat load: a between-population analysis. Am J Phys Anthropol 119(3):276–282.

Wennerholm UB, Bergh C, Hamberger L, Lundin K, Nilsson L, Wikland M, et al. 2000. Incidence of congenital malformations in children born after ICSI. Hum Reprod 15:944–948.

Werler MW, Louik C, Shapiro S, Mitchell AA. 1996. Prepregnant weight in relation to risk of neural tube defects. J Am Med Assoc 275:1089–1092.

Westergaard HB, Johansen AM, Erb K, Andersen AN. 1999. Danish national *in-vitro* fertilization registry 1994 and 1995: a controlled study of births, malformations and cytogenetic findings. Hum Reprod 14:1896–1902.

WHO. 1995. Maternal Anthropometry and Pregnancy Outcomes: A WHO Collaborative Study. Bull World Health Organ 73(suppl):1–98.

Wilkinson CF, Lamb JC. 1999. The potential health effects of phthalate esters in children's toys: a review and risk assessment. Regul Toxicol Pharmacol 30:140–155.

Wong WY, Thomas CMG, Merkus HMWM, Zielhuis GA, Doesburg WH, Steegers-Theunissen RPM. 2000. Cigarette smoking and the risk of male factor subfertility: minor association between cotinine in seminal plasma and semen morphology. Fertil Steril 74:930–935.

Xiao G, Pan C, Cai Y, Lin H, Fu Z. 1999. Effect of benzene, toluene, xylene on semen quality of exposed workers. Chin Med J 112:709–712.

Xu B, Chia SE, Tsakok M, Ong CN. 1993. Trace elements in blood and seminal plasma and their relationship to sperm quality. Reprod Toxicol 7:613–618.

Yazigi RA, Odem, RR, Polakoski KL. 1991. Demonstration of specific binding of cocaine to human spermatozoa. JAMA 266:1956–1959.

Younglai EV, Foster WG, Hughes EG, Trim K, Jarrell JF. 2002. Levels of environmental contaminants in human follicular fluid, serum, and seminal plasma of couples undergoing *in vitro* fertilization. Arch Environ Contam Toxicol 43:121–126.

Zenzes MT, Puy LA, Bielecki R, Reed TE. 1999. Detection of benzo[*a*] pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. Mol Hum Reprod 5:125–131.

Zhou LM, Yang WW, Hua JZ, Deng CQ, Tao X, Stoltzfus RJ. 1998. Relation of hemoglobin measured at different times in pregnancy to preterm birth and low birth weight in Shanghai, China. Am J Epidemiol 148(10):998–1006.