Chapter 8
Reproductive and Developmental Effects

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Introduction

Health professionals have long considered exposure to tobacco smoke harmful to reproduction, affecting aspects from fertility and pregnancy outcome to fetal and child development. Tobacco smoke contains thousands of compounds, some of which are known to have toxic effects on reproductive health, such as carbon monoxide (CO), nicotine, and metals. Along with more than four million births in the United States annually, 10 to 20 percent of pregnancies end in miscarriage or stillbirth before delivery, and another 10 percent of couples who want to conceive a child experience infertility or reduced fertility. In 2007, 17.4 percent of all women and approximately 19 percent of women of reproductive age (18 through 44 years) smoked cigarettes (Centers for Disease Control and Prevention [CDC] 2008). Smoking rates among women of reproductive age vary by other factors such as education, race, and geographic area, ranging from about 10 percent in Utah to nearly 30 percent in Kentucky and West Virginia (CDC 2005). From 2002 to 2005, 17.3 percent of pregnant women reported smoking cigarettes in the past month (*NSDUH Report* 2007). CDC's Pregnancy Risk Assessment Monitoring System is an ongoing, population-based surveillance system designed to identify and monitor selected self-reported maternal behaviors and experiences that occur before, during, and after pregnancy among women who deliver a live infant. In 2002, the prevalence of smoking in the three months before pregnancy ranged from 13.6 percent (Utah) to 37.0 percent (West Virginia); in the last three months of pregnancy, from 6.8 percent (Utah) to 25.3 percent (West Virginia); and after pregnancy, from 9.0 percent (Utah) to 33.7 percent (West Virginia) (Williams et al. 2006). Prevalence of smoking is generally higher among men. In 2003, 24.1 percent reported smoking and prevalence was higher among younger men than among older men. This chapter examines reproductive and developmental outcomes, although the term "reproductive" may be used generally to describe both, in relation to smoking.

The reproductive endpoints include aspects affecting a person's ability to conceive a child, such as menstrual cycle function, semen quality, fertility, and menopause, in addition to complications of pregnancy, such as miscarriage, ectopic pregnancy, and preterm delivery. Developmental endpoints that affect child health status include birth weight, congenital anomalies, and perinatal and infant deaths—especially sudden infant deaths and sudden unexplained infant deaths which have been associated with exposure to secondhand smoke—and they extend into childhood with neurobehavioral endpoints and puberty. Previous Surgeon General's reports have examined epidemiologic data for most of these endpoints. This chapter cites conclusions from those earlier reports, examines in more detail endpoints for which the evidence was not sufficient to establish causality, and provides an updated review of the epidemiologic literature for these endpoints. Other sections explore the possible biologic basis for an effect of smoking on reproduction and development from the pathophysiological levels to the cellular and genetic levels.

When studying the reproductive effects of smoking in humans, there are several exposure issues to bear in mind. Most studies have examined the effects of active smoking on fertility or pregnancy. For the past decade, interest has also increased in the effects of secondhand exposure to tobacco smoke, so these studies are mentioned when available (U.S. Department of Health and Human Services [USDHHS] 2006). Because smoking rates have declined, persons who are involuntarily exposed to tobacco smoke probably now outnumber active smokers. Thus, many nonsmokers are exposed to some of the same toxins to which smokers are exposed. The problem of involuntary exposure may be particularly pervasive for women who stop smoking during pregnancy. They may live with partners or family members who continue to smoke, so the potential still exists for exposure to tobacco smoke in the household. Such an exposure may also occur in the workplace. However, local, state, and federal laws against smoking in the workplace have led to a decline in this type of exposure. The critical exposure periods may be very specific for certain pregnancy outcomes or congenital anomalies, but most epidemiologic studies do not seek such detailed information about exposure to tobacco smoke. For endpoints of child development, postnatal exposure to tobacco smoke may also be important but difficult to separate from prenatal exposure, because the two are correlated.

Current smoking may be assessed for reproductive endpoints such as fertility, but this timing may not reflect exposure during the critical period when fertility began if the woman has stopped smoking as a result of ongoing fertility problems. Fertility may also be affected by her partner's smoking, either directly or indirectly as exposure to secondhand smoke. Research shows the long-lasting effects of prenatal exposures on later health, even in adulthood. Thus, age at puberty, fertility, or even maintenance of a pregnancy may be affected by in utero exposure to tobacco smoke, but this relationship is rarely studied. For age at menopause, patterns of exposure to tobacco smoke over a lifetime may be important.

Review of Epidemiologic Literature on Smoking

Reproductive Endpoints

Menstrual Function, Menarche, and Menopause

Menstrual Cycling

The effects of exogenous exposures on menstrual function have become the focus of much research. Studies of these effects are hindered because cyclic patterns of menstruation vary and do not have one well-defined health endpoint. For example, some menstrual disturbances such as irregularity do not have standard definitions, and others, such as dysmenorrhea (painful menstruation), may be subjective. However, menstrual morbidity has a significant impact on women's health and economics (e.g., physician visits and time lost from work) (Harlow and Ephross 1995). Furthermore, menstrual cycle patterns are a useful marker of ovarian function and reproductive health and may affect risks of chronic disease. The 2004 Surgeon General's report on the health consequences of smoking did not examine menstrual function or menopause, but the 2001 report on women and smoking reached suggestive conclusions that are expanded upon here (USDHHS 2001, 2004).

Beginning in the 1960s, numerous studies have examined menstrual function in relation to smoking, but most were focused on dysmenorrhea or other self-reported symptoms. As summarized in the 2001 Surgeon General's report on women and smoking (USDHHS 2001), the prevalence of dysmenorrhea was increased with current smoking, with intermediate effects among former smokers (Brown et al. 1988; Parazzini et al. 1994; Harlow and Park 1996; Mishra et al. 2000), but not in all studies (Andersch and Milsom 1982). A Chinese study of exposure to secondhand smoke in nonsmoking women reported an adjusted risk for dysmenorrhea that increased with higher exposure levels (Chen et al. 2000). In examining multiple endpoints or symptoms, a community survey in Los Angeles, California, revealed that the prevalence of physician-attended menstrual disorders (e.g., dysmenorrhea and oligomenorrhea) was higher among heavy smokers (≥15 cigarettes per day) than among nonsmokers (Sloss and Frerichs 1983). A postal survey in England found that compared with nonsmokers, smokers more frequently reported six of seven aspects of "abnormal" menstruation, including frequent, short, or irregular periods and prolonged and heavy bleeding (Brown et al. 1988).

Similarly, other worldwide studies have reported higher risks of multiple symptoms, including premenstrual tension, heavy periods, severe pain, and frequent and irregular periods among smokers, especially heavy smokers (Kritz-Silverstein et al. 1999; Mishra et al. 2000). Additional studies reported increased risks of short and/or irregular cycles among smokers, with some dose-response relationships observed (Kato et al. 1999; Rowland et al. 2002). Using prospective menstrual diaries to improve ascertainment, Hornsby and colleagues (1998) found more reporting of dysmenorrhea, an increased daily amount of bleeding, and a shorter duration of bleeding among smokers. The findings suggested that heavy smokers (>10 cigarettes per day) had irregularity or greater variability in cycle length than did nonsmokers. However, the study had limited power to examine higher smoking levels, and the study sample was selective in that the participants' mothers had participated in a clinical trial of diethylstilbestrol while pregnant with them.

Other studies have assessed menstrual cycle parameters by measuring hormone levels to define lengths of phases in the cycle. A small study noted cycles of heavy smokers that were, on average, 1.6 days shorter than cycles of nonsmokers, and the mean follicular phase was shorter by 1.4 days (Zumoff et al. 1990). A study based on diaries and daily measurement of urinary levels of hormone metabolites reported that heavy smoking (≥20 cigarettes per day) was also associated with menstrual cycle lengths that were shorter by 2.6 days and more variable than those of nonsmokers (Windham et al. 1999b). The shortening of the cycle occurred primarily during the follicular phase. The findings also suggested an increased risk of a short luteal phase (<11 days) and anovulation, but the confidence intervals (CIs) for these endpoints were wide and not significant. The mean duration of bleeding in smokers was not different. Another study, based on diaries of workers in the semiconductor industry, as well as levels of hormone metabolites, found little difference in cycle length among smokers compared with nonsmokers (Liu et al. 2004a). However, this finding was modified by age: shorter follicular phases were associated with smoking only among women older than 35 years of age. The data also revealed a nonsignificant increase in risk of anovulation among smokers. Dose-response relationships were not examined (Liu et al. 2004b).

Alterations in menstrual cycle function may have several ramifications, including a burden on the health care system. Dysmenorrhea may lead to a loss of work productivity. Women with variable cycle lengths may have difficulty trying to conceive, because the timing of ovulation is less predictable. Anovulation has an obvious relevance for time to conception or fertility. Cycles that

are shortened during the follicular phase might indicate abnormal folliculogenesis and ovum maturation. A short luteal phase may indicate a progesterone response that is inadequate for implantation and maintenance of the trophoblast. Studies have implicated a luteal phase defect as a cause of infertility as well as a cause of recurrent spontaneous abortion (SAB) (Regan et al. 1990; Tulppala et al. 1991). These effects are consistent with evidence for association of smoking with decreased fertility (see "Fertility" later in this chapter). Women with short menstrual cycles may also be at a higher risk of breast cancer (Kelsey et al. 1993).

Reproductive Life Span—Menarche to Menopause

Smoking may also affect the duration of menstrual cycling (reproductive life span). The 2001 Surgeon General's report on women and smoking summarized numerous studies that consistently found a younger age at natural menopause among women who smoked than that for nonsmokers (USDHHS 2001). The studies also concluded that smokers may have more menopausal symptoms. In an earlier meta-analysis, the difference in the mean age at natural menopause ranged from 0.8 to 1.7 years (Midgette and Baron 1990). This same meta-analysis showed a prevalence ratio for being postmenopausal that was nearly doubled among current smokers versus lifetime nonsmokers, with dose-response trends by the number of cigarettes smoked. Later studies confirmed these findings (Cooper et al. 1999; Harlow and Signorello 2000; Meschia et al. 2000; Brett and Cooper 2003). One study reported a decrease in mean age at natural menopause with current active smoking but did not find an association among former smokers or with exposure to secondhand smoke (Cooper et al. 1999). However, two studies that were more briefly described found an earlier age at menopause with exposure to secondhand tobacco smoke (Everson et al. 1986; Tajtakova et al. 1990). In a population-based study in the United States, smoking was weakly associated with transition to menopausal status and strongly associated with postmenopausal status (Brett and Cooper 2003). This finding led the authors to suggest that the menopausal transition period may be shortened in smokers.

On the other end of the spectrum, some studies have examined age at menarche (start of menstrual periods) in relation to parental smoking. On the basis of data from a longitudinal birth cohort study, daughters whose mothers had smoked heavily during pregnancy had an earlier mean age at menarche by several months (Windham et al. 2004). This effect was greater among non-Whites than among Whites. Two studies from Poland reported a younger age at menarche for daughters of smoking mothers than that for daughters of nonsmoking mothers (Kolasa 1997; Kolasa et al. 1998). A retrospective study of teachers found a slightly higher risk of early menarche among women who reported that during their childhoods, their parents had smoked at home (Reynolds et al. 2004). These later studies primarily examined exposure to secondhand smoke, and the timing with respect to puberty was not established. However, mothers who smoked postnatally, especially before smoking was socially prohibited, may have smoked during pregnancy as well. Windham and colleagues (2004) showed that girls with high prenatal and childhood exposure to secondhand smoke had the earliest mean age at menarche. One study examined the effects of parental smoking on puberty in both boys and girls and reported earlier pubertal milestones in boys whose mothers had smoked during pregnancy, but not in girls. However, the study had such small numbers that the power to examine age at menarche was insufficient (Fried et al. 2001).

Changes that affect the reproductive life span can have an impact on other aspects of a woman's health. Shorter cycles may lead to a more rapid depletion of oocytes, shortening the reproductive life span and leading to earlier menopause (Whelan et al. 1990; Bromberger et al. 1997). Early menopause is associated with other hormone-related health problems such as osteoporosis and cardiovascular disease (Harlow and Ephross 1995; Sowers and La Pietra 1995; Cooper and Sandler 1998). Early menarche or puberty may lead to psychosocial problems, adolescent pregnancy and attendant risks, other adverse reproductive outcomes, and breast cancer (Hardy et al. 1978; Liestol 1980; MacMahon et al. 1982a; Martin et al. 1983; Sandler et al. 1984; Wilson et al. 1994; Ge et al. 1996; He and Karlberg 2001).

Fertility

Fertility is an endpoint that is difficult to compare across studies, because no standard definition exists. Fecundity refers to the biologic ability to conceive, given unprotected intercourse, and depends on the reproductive capacity of both sexual partners. The clinical definition of infertility in the United States usually connotes lack of conception after one year of unprotected intercourse during the fertile phase. However, couples who delay childbearing may seek treatment before one year, which further complicates studies. Subfertility refers to any form of reduced fertility in couples trying to conceive, and one way to study it is by measuring time to conception or pregnancy. One commentary indicated that about 20 percent of couples experience subfertility, defined as the inability to conceive within six months (Gnoth et al. 2005). About 50 percent of these couples conceive in the next six months, leaving

10 percent of couples that match the clinical definition of infertility. Another 50 percent will likely conceive spontaneously in the next three years, leaving 5 percent infertile. Smoking affects fertility in men and women, as well as the success of in vitro fertilization (IVF).

Fertility in Females

Numerous studies have found associations of smoking with reduced fertility. The 2001 Surgeon General's report concluded that "women who smoke have increased risks for conception delay and for both primary and secondary infertility" (USDHHS 2001, p. 14). The 2004 Surgeon General's report also reviewed the literature and concluded that "the evidence is sufficient to infer a causal relationship between smoking and reduced fertility in women" (USDHHS 2004, p. 7). Other reviews have found consistent decrements in fertility associated with smoking, as well as evidence for dose-response trends. In a meta-analysis of data from 12 studies, the odds ratio (OR) was 1.6 (95 percent CI, 1.3–1.9) for infertility among smokers (Augood et al. 1998). Furthermore, meta-analyses of data on IVF treatment indicated a reduction in fecundity (conception rate per cycle) among women smokers ranging from 0.57 (95 percent CI, 0.42–0.78) to 0.66 (95 percent CI, 0.49–0.88) (Hughes and Brennan 1996; Augood et al. 1998). In a later qualitative review of 22 studies, all but 3 of the studies indicated a detrimental effect of smoking on female fecundity (Wilks and Hay 2004). The Practice Committee of the American Society for Reproductive Medicine (PCASRM) also issued a statement strongly supporting evidence for an association between smoking and infertility, estimating that 13 percent of infertility may be attributable to smoking (PCASRM 2004).

Several studies also examined the effects of exposure to secondhand tobacco smoke on fertility, but these effects may be difficult to separate from the direct effects of smoking on the partner's fecundity. Researchers have reviewed these studies and found suggestive but inconsistent results (National Cancer Institute [NCI] 1999; USDHHS 2001, 2006), so there are insufficient data to reach conclusions. A study published since two of those reviews examined the effects of exposure to mainstream and sidestream smoke on IVF outcomes. The researchers found that implantation and pregnancy rates were reduced by about one-half from both types of exposure to tobacco smoke among smokers compared with nonsmokers (Neal et al. 2005). The investigators noted that the association of exposure to sidestream smoke may reflect some direct effect of smoking on the male partners. However, they claimed that this could not explain the entire effect in that they observed no difference in implantation and pregnancy rates by the partner's smoking level.

Intriguingly, some studies have also examined the effects of in utero exposure to maternal smoking on later fertility or fecundability. One study found a significantly reduced likelihood of conception in smokers versus nonsmokers (fecundability ratio [FR]) of 0.5 (Weinberg et al. 1989); another study found a slightly reduced FR of 0.9 (Wilcox et al. 1989); and a third study found a slightly increased FR of 1.1 but also observed no effect of active smoking among the women (Schwingl 1992). In the range of these studies, a Danish study reported FRs for in utero exposure to cigarette smoking of 0.70 among adult nonsmokers and 0.53 among smokers, as well as 0.67 for female smokers with no in utero exposure, all compared with that for nonsmokers who had no in utero exposure (Jensen et al. 1998b). This study also found a similarly decreased fecundability in males exposed to smoking in utero.

Effects on Semen Quality and Male Fertility

For decades, epidemiologic studies have investigated the effects of cigarette smoking on semen quality, because of its relationship to male fertility (Campana et al. 1996; Eggert-Kruse et al. 1996; Bonde et al. 1998; Chia et al. 2000), although this relationship is not always predictive (Polansky and Lamb 1988; Guzick et al. 2001). Semen quality is measured by seminal plasma constituents and sperm cell characteristics such as ejaculate volume, sperm count, sperm motility, and sperm morphology. The *World Health Organization (WHO) Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction* (WHO 1980, 1987, 1992, 1999b) provides reference values for many semen parameters associated with fertility. A number of epidemiologic studies of smoking effects have used WHO reference values to categorize participants by their fertility status. Reproductive hormones are important determinants of normal sperm production and male sexual function and have thus also been studied in relation to exposure to cigarette smoking (see "Endocrine System" later in this chapter).

The 2004 Surgeon General's report reviewed the published epidemiologic literature evaluating semen quality and fertility and concluded that "the evidence is inadequate to infer the presence or absence of a causal relationship between active smoking and sperm quality" (USDHHS 2004, p. 28). However, "the evidence suggests that smokers may have decreased semen volume and sperm number and increased abnormal [morphologic] forms, although any clinical relevance of these findings is not clear" (USDHHS 2004, p. 534). A stronger causal statement could not be made at that time because of variability in published findings across studies. Reviews on this topic (Mattison 1982; Stillman et al. 1986; Little and

Vainio 1994; Vine 1996; Marinelli et al. 2004; PCASRM 2004) and one meta-analysis of data from 20 studies covering 1966–1992 (Vine et al. 1994) attribute this variation in the findings across studies to weaknesses in study designs. These weaknesses include a failure to adjust for potential confounders, small sample sizes, inadequate exposure assessments, and the use of fertility clinic populations.

Subsequent to the 2004 Surgeon General's report (USDHHS 2004), the PCASRM review (2004), and the review by Marinelli and colleagues (2004), some publications have strengthened the evidence for decrements in adult semen quality and fertility associated with exposure to tobacco smoke either prenatally or in adulthood (Table 8.1). Both studies in Table 8.1 that evaluated adult semen quality after in utero exposure found decrements in sperm concentration (Storgaard et al. 2003; Jensen et al. 2004). Studies conducted in clinical settings using assisted reproductive technology with extremely sensitive measures of fertilization and very early pregnancy loss also reported adverse effects from paternal smoking. Some studies had low power for detecting effects, but one of the largest studies, which was based on a retrospective review of records, did not find independent effects of cigarette smoking. However, the researchers reported a reduction in seminal volume, sperm concentration, and the percentage of motile sperm in men who smoked and consumed alcohol, behaviors that tend to be correlated (Martini et al. 2004). Another study based on a retrospective review of medical records for exposure assessments could not include more than 46 percent of the medical records initially reviewed, because of insufficient data, particularly data related to smoking (Ozgur et al. 2005). Although the study found no significant differences in sperm density, normal sperm tail morphology decreased or abnormal forms increased.

Spermatogenesis in the adult testes depends on a hormonal milieu that is temporally and compartmentally specific (e.g., seminiferous tubule, interstitial space, and epididymis). Thus, factors likely to be responsible for the decrements seen in semen quality and male fertility are constituents of cigarette smoke that influence the normal development and adult function of the hypothalamus and pituitary gland or that influence the differentiation, development, and adult function of Leydig and/or Sertoli cells that secrete hormones (see "Endocrine System" later in this chapter). Support for the contribution of smoking effects on hormonal factors to male fertility is still evolving (Meikle et al. 1989; Michnovicz et al. 1989; Sofikitis et al. 1995; Chapin et al. 2004). Other potential mechanisms of smoking for male infertility include effects on the sperm plasma membrane (Belcheva et al. 2004) and tobacco-related damage to DNA and/or chromosomes in gametes (PCASRM 2004) (see "Genetic Damage to Sperm" later in this chapter). Studies have associated in utero exposure to polycyclic aromatic hydrocarbons (PAHs) in tobacco smoke with adverse effects on male fertility and on testes (see "Polycyclic Aromatic Hydrocarbons" later in this chapter). Additional targeted research in human populations with use of molecular laboratory tools will help to elucidate the mechanisms underlying these observations.

Study designs have not addressed the timing of exposures in relation to sperm cell maturation in the testes, the formation and secretion of fluids contributed by accessory organs outside the testes, and events during fertilization. This information is critical to unraveling mechanisms of the toxic effects of tobacco smoke, determining whether these exposures result in long-term risks to male reproductive health, and documenting the benefits of smoking cessation. For example, if spermatogonial stem cells prove to be the cells most sensitive to exposure to tobacco smoke, then long-term consequences might be observed even after smoking cessation. However, if mechanisms of toxic effects relate primarily to effects on epididymal sperm or to direct effects on mature sperm in the ejaculate from seminal plasma constituents, then smoking cessation could result in immediate benefits.

Pregnancy Complications

This section addresses a variety of complications that may occur during pregnancy. These complications primarily represent difficulties the pregnant mother may experience trying to maintain a healthy pregnancy, but there also may be wide-ranging effects on the health of the fetus or the offspring. In general, these conditions may be influenced by maternal age, reproductive history, and medical history or conditions affecting the maternal endocrine or immune systems, uterine structure, and cardiovascular system, among others. Exogenous exposures may also play a role in causing or exacerbating these conditions. This section briefly presents the etiology of these complications and puts potential smoking-induced mechanisms into context. (For details on the mechanisms of these complications, see "Pathophysiological and Cellular and Molecular Mechanisms of Smoking" later in this chapter.)

Spontaneous Abortion

SAB is typically defined as the involuntary termination of an intrauterine pregnancy before 20 weeks of gestation. Studies have reported recognized SABs in approximately 12 percent of pregnancies, and most occur

Table 8.1 Association of adult cigarette smoking and in utero exposure to cigarette smoke with semen parameters and fertility in adults[a]

Study	Population	Study period	Definition of smoking
	Effects from adult smoking		
Chia et al. 2000	240 fertile and 218 infertile men from obstetric and andrology clinic populations	NR	Self-reported number of cigarettes/day and smoking history
Trummer et al. 2002	260 smokers, 70 former smokers, and 258 nonsmokers from infertility clinic population	1993–2000	Self-reported number of cigarettes/day
Gaspari et al. 2003	69 current smokers, 22 former smokers, and 88 lifetime nonsmokers from infertility clinic population, 75 with 1-year follow-up for fertility	2001	Self-reported number of cigarettes/day and smoking history
Künzle et al. 2003	655 smokers and 1,131 nonsmokers from infertility clinic population	1991–1997	Self-reported >1 cigarette/day
Loft et al. 2003	225 women with first pregnancy, 74 male smokers, and 151 male nonsmokers	1992–1994	Self-reported number of cigarettes/day
Zitzmann et al. 2003	48 IVF couples and 153 ICSI couples	1999–2001	Self-reported lifetime nonsmoker or smoker (≥5 cigarettes/day for 2 years)
Chen et al. 2004	22 smokers, 57 former smokers, and 227 nonsmokers from infertility clinic population in study of seasonal effects and environmental exposures	2000–2002	Self-reported current smoker, former smoker, or lifetime nonsmoker
Jurasović et al. 2004	61 smokers and 62 nonsmokers from infertility clinic population	NR	Self-reported number of cigarettes/day
Martini et al. 2004	3,430 nonsmokers, 422 light smokers, and 124 heavy smokers from infertility clinic population	1990–1999	Retrospective review of records for cigarettes/day: 0, 1–20, >20

Findings	OR, RR, or % (95% CI or p value)	Comments
	Effects from adult smoking	
• Smoking predictive of risk of infertility	Infertility in smokers compared with nonsmokers = 2.96 (1.98–4.42)	None
• NS difference in density, motility, and morphology	NR	None
• PAH-DNA adducts in sperm associated with infertility but not cigarette smoking • PAH-DNA adducts associated directly with abnormal forms of sperm and inversely with asthenospermia	Smokers = 1.58 Nonsmokers = 1.62 in relative staining intensity (NS) PAH-DNA adducts associated with abnormal head (r = 0.3), abnormal neck (r = -0.2), and abnormal physiological forms (r = -0.2)	None
• Lower sperm density, total motile cells, and citrate concentration • Fewer morphologically normal cells • Higher pH • No significant difference in volume, vitality, and fructose concentration	Decreased density by 15.3%, p = 0.0001 Decreased total motile cells by 16.6%, p = 0.002 Decreased normal forms by 10.6%, p = 0.0007 Decreased citrate concentration by 22.4%, p = 0.007	Correlation of alcohol intake and smoking (p = 0.002) after removing heavy drinkers because moderate alcohol intake has not been associated with infertility; no adjustment
• Likelihood of pregnancy in a menstrual cycle inversely associated with 8-oxo-dG concentrations, but levels not associated with smoking status	Correlation of smoking and 8-oxo-dG concentrations Spearman correlation 0.02 (NS)	Adequate semen count required to extract enough DNA for bioassay because variance by smoking may bias findings
• Paternal smoking predictive of fertilization in IVF but not in ICSI • Paternal smoking predictive of clinical pregnancy in ICSI and IVF • Increased progressive motility and decreased normal forms of sperm among smokers	ICSI pregnancy failures in partners of male smokers compared with partners of nonsmokers 2.95 (1.32–6.59) IVF pregnancy failures in partners of male smokers compared with partners of nonsmokers 2.65 (1.33–5.30)	None
• NS differences in concentration, motility, and morphology of sperm	NS 20% decrease in sperm density in current smokers compared with lifetime nonsmokers	Small number of smokers
• Decreased blood prolactin • Positive correlation with blood cadmium • NS differences in concentration, motility, and morphology of sperm	Decreased blood prolactin (p = 0.02) Correlations with blood: Cadmium, +0.80 (p <0.001) Selenium, -0.26 (p <0.01) Selenium-glutathione peroxidase, -0.30 (p <0.001)	None
• NS differences in concentration, motility, and morphology of sperm across categories of smoking independent of alcohol intake	Significantly decreased density by 11%, decreased total motile sperm 10%, decreased rapid sperm by 14% for men who smoked >1 cigarette and drank ≤52 grams of ethanol/day compared with no smoking or drinking	Retrospective review of record with no exposure validation measures

Table 8.1 Continued

Study	Population	Study period	Definition of smoking
Ozgur et al. 2005	116 heavy smokers, 82 light smokers, and 98 nonsmokers from infertility clinic patients	2000–2002	Retrospective review of records for cigarettes/day: 0, 1–19, ≥20
Effects during adulthood of in utero exposure to cigarette smoke			
Storgaard et al. 2003	265 Danish men identified from population-based birth registries	1999–2000	Maternal recall of smoking during pregnancy: 0, 1–10, 11–20, >20 cigarettes/day
Jensen et al. 2004	1,770 military inductees from Denmark, Estonia, Finland, Lithuania, and Norway	1996–1999	Maternal recall of maternal and paternal smoking during pregnancy: yes, no

Note: **8-oxo-dG** = 7-hydroxy-8-oxo-2′-deoxyguanosine; **CI** = confidence interval; **ICSI** = intracytoplasmic sperm injection; **IVF** = in vitro fertilization; **NR** = not reported; **NS** = not statistically significant; **OR** = odds ratio; **PAH** = polycyclic aromatic hydrocarbon; **RR** = rate ratio.
[a]Not included in the 2004 Surgeon General's report on the health consequences of smoking.

before 12 weeks of gestation (Regan et al. 1989). However, very early pregnancy loss may go unrecognized and/or unreported. An estimated 30 to 45 percent of conceptions actually end in pregnancy loss (Wilcox et al. 1988; Eskenazi et al. 1995). Studies of tissue from SABs suggest that 50 to 80 percent of losses have an abnormal karyotype, depending on the mother's age and the gestational age at the time of the loss; a higher proportion of abnormalities is found in losses at earlier gestational ages (Kajii et al. 1980; Hogge et al. 2003; Philipp et al. 2003). In addition to fetal abnormalities, other factors that likely contribute to SAB include maternal anatomical abnormalities of the uterus, immunologic disturbances, thrombotic disorders, and endocrine abnormalities (Christianson 1979; Cramer and Wise 2000; Regan and Rai 2000). Infections may also play a role, but data are limited and inconsistent (Cramer and Wise 2000; McDonald and Chambers 2000; Matovina et al. 2004).

Several studies have demonstrated a moderate association between smoking and SAB (DiFranza and Lew 1995). The 2004 Surgeon General's report on the health consequences of smoking found the evidence suggestive but not sufficient to infer a causal relationship between smoking and SAB (USDHHS 2004). However, numerous studies are available since the handful reviewed at that time (Table 8.2), and most show positive associations. These results represent both retrospective and prospective study designs from a number of different countries, with adjustment for various confounders. One study found an association with amount smoked before pregnancy (Nielsen et al. 2006), and another reported an association among former smokers (Mishra et al. 2000), but others did not observe these associations (Chatenoud et al. 1998; Wisborg et al. 2003). The later study had a very low SAB rate of approximately 1 percent, so many SABs were likely missed, particularly early in pregnancy. Two studies used a measurement of cotinine to verify exposure to tobacco smoke and found relatively high risks of SAB (Ness et al. 1999; George et al. 2006). Estimates of the increase in risk of SAB from smoking are 30 to 100 percent, some in a dose-response pattern (Kline et al. 1977; Chatenoud et al. 1998; USDHHS 2004; George et al. 2006; Nielsen et al. 2006). In a meta-analysis of data from 13 studies, the pooled ORs for SAB in smokers were 1.24 (95 percent CI, 1.19–1.30) for cohort studies and 1.32 (95 percent CI, 1.18–1.48) for case-control studies (DiFranza and Lew 1995). In one study, the association with smoking appeared stronger in chromosomally normal SABs (Kline

Findings	OR, RR, or % (95% CI or p value)	Comments
• Increased rapidly motile cells among smokers of ≥20 compared with 1–19 cigarettes/day • Increased coiled-tail defects among smokers • NS difference in density and total motile cells in nonsmokers compared with heavy smokers	Decreased rapidly motile cells by 30% at ≥20 compared with 1–19 cigarettes/day (p <0.05) Decreased normal tail morphology by 18% for smokers of ≥20 cigarettes/day compared with nonsmokers (p <0.05)	Retrospective review of records with no exposure validation measures; missing data on more than 46% of records
Effects during adulthood of in utero exposure to cigarette smoke		
• In utero exposure to >10 cigarettes/day associated with lower sperm density (48%), lower inhibin B, and higher follicle-stimulating hormone • No dose-response relationship or significant change in other semen parameters or hormones	Decreased sperm concentration by 48% (-69 to -11%) at exposure of >10 cigarettes/day	None
• Lower sperm concentration and total sperm count among those exposed to smoking • NS difference in sperm motility, morphology, or testis size	Decreased sperm concentration by 20.1% (6.8–33.5%) Decreased total sperm count by 24.5% (9.5–39.5%)	None

et al. 1995), but another study did not find a differential effect by chromosomal abnormality (George et al. 2006).

In examining exposure to secondhand smoke, the 2006 Surgeon General's report on the health consequences of involuntary exposure to tobacco smoke concluded that the evidence was "inadequate to infer the presence or absence of a causal relationship" with SAB, on the basis of a few studies with inconsistent results (USDHHS 2006, p. 13). Since then, two studies have found an association with secondhand smoke on the order of 60 to 80 percent, one with a biomarker of cotinine (George et al. 2006) and one that examined very early fetal loss (Venners et al. 2004). Another study did not find an association in examining only paternal smoking (Chatenoud et al. 1998), and one found an effect of exposure to secondhand smoke at either home or work only among women who also consumed alcohol or high amounts of caffeine (Windham et al. 1999c).

Proposed mechanisms for an effect from tobacco smoke include fetal hypoxia from exposure to CO, vasoconstrictive and antimetabolic effects resulting in placental insufficiency and the subsequent death of the embryo or fetus (PCASRM 2004), and direct toxic effects of constituents of cigarette smoke.

Ectopic Pregnancy

Ectopic pregnancy occurs when a fertilized egg is implanted outside the uterus, usually within the fallopian tube. It is estimated to occur in 1 to 2 percent of pregnancies (Chow et al. 1987; Goldner et al. 1993; Van Den Eeden et al. 2005) and accounts for approximately 6 percent of pregnancy-related deaths in the United States (Berg et al. 2003; Chang et al. 2003). Factors associated with ectopic pregnancy include a history of sexually transmitted diseases and pelvic inflammatory disease, increased number of sexual partners, maternal age, history of SAB, history of surgical procedures affecting the fallopian tubes, previous use of an intrauterine device, and vaginal douching (Kendrick et al. 1997; Pisarska et al. 1998; Bouyer et al. 2003). Affected women are at increased risk of infertility and recurrent ectopic pregnancy in subsequent pregnancies (Chow et al. 1987; Coste et al. 1991; Washington and Katz 1993; Skjeldestad et al. 1998), as would be expected among women with tubal damage.

The 2004 Surgeon General's report found the evidence suggestive but not sufficient to infer a causal relationship between smoking and ectopic pregnancy (USDHHS 2004). A number of studies have associated

Table 8.2 Association between maternal smoking and spontaneous abortion (SAB), 1998–2006

Study	Design/population	Definition of smoking	OR (95% CI)	Comments
Chatenoud et al. 1998	Case-control study 782 cases of SAB by 12 weeks' gestation, confirmed pathologic examination 1,543 women who delivered term healthy infants in same hospitals as cases Italy	Lifetime nonsmokers vs. women who smoked during pregnancy None, 1–4, 5–9, ≥10 cigarettes/day Any smoking ≥10 cigarettes/day	 1.3 (1.0–1.6) 1.4 (1.0–2.1)	No association with smoking before pregnancy or with paternal smoking; adjustment for center, age, marital status, history of SAB, nausea, and use of alcohol and coffee
Ness et al. 1999	Case-control study 400 adolescents or women with SAB <22 weeks' gestation at initial visit or during follow-up 570 adolescents or women with pregnancies beyond 22 weeks' gestation seeking care at inner-city emergency room Low SES population Pennsylvania	Urinary cotinine >500 ng/mL used to identify heavy smokers Self-reported smoking during pregnancy	 1.8 (1.3–2.6) 1.4 (1.0–1.9) Crude data	Adjustment for use of cocaine, marijuana, and alcohol, and living with partner, weeks of gestation at interview, and prenatal care; stronger among SABs during follow-up (OR 2.4)
Windham et al. 1999c	Prospective study of women seeking prenatal care at large health maintenance organization (Kaiser) by 12 weeks' gestation 499 SABs by 20 weeks' gestation, from medical records 5,144 pregnancies after excluding therapeutic abortions California	Smoking at 8 weeks of gestation and amount vs. nonsmoking ≥5 cigarettes/day Spouse smoking status Hours of exposure to SHS at home and work for nonsmokers	 1.3 (0.9–1.9) No association No association, unless consumption of caffeine or alcohol OR ~3 for any exposure to SHS	Adjustment for age, previous fetal loss, gestational age at interview, use of alcohol and caffeine; effect modification with high use of caffeine or alcohol (>3 drinks/week)
Mishra et al. 2000	Prospective study 14,779 women (aged 18–23 years), from baseline survey Self-reported SABs (≥1) Australia	Lifetime nonsmokers vs. current and former smokers Amount (cigarettes/day) Former smokers Current smokers 1–9 10–19 ≥20	 1.6 (1.3–2.2) 1.7 (1.1–2.5) 1.6 (1.6–2.3) 2.0 (1.5–2.8)	Adjustment for parity, therapeutic abortion, age, education, marital status, and area of residence; higher risk for smoking initiation at younger age

Table 8.2 Continued

Study	Design/population	Definition of smoking	OR (95% CI)	Comments
Rasch 2003	Case-control study 330 women with SAB ascertained by evacuation procedure at hospital, 6–16 weeks' gestation 1,168 pregnant women receiving prenatal care in weeks 6–16 Denmark	Smoking during pregnancy and amount vs. none ≥20 cigarettes/day vs. none	 0.95 (0.4–2.2) Adjusted data 2.2 (1.1–4.8) Crude data	Large effect of adjustment for age, parity, occupation, and use of alcohol and caffeine; dose-response relationship before adjustment; 40% of original eligible subjects not included in study
Wisborg et al. 2003	Prospective study among women seeking prenatal care 24,608 pregnant women who completed questionnaire by 28 weeks' gestation 321 SABs by 28 weeks (104 first trimester, 217 second trimester) from Danish patient registry Denmark	Smoking before or during pregnancy (and amount) vs. nonsmokers ≥10 cigarettes/day during pregnancy for second trimester SABs First trimester	 0.88 (0.59–1.31) 0.92 (0.55–1.54)	Crude estimates; adjustment made no difference; very low SAB rate (~1%), especially with extended definition (28 weeks); median entry time was second trimester, so most earlier SABs were missed
George et al. 2006	Case-control population-based study 463 women with SAB at 6–12 weeks' gestation, interviewed after SAB (most within 2 weeks) 864 pregnant women with viable pregnancy, matched to cases by gestational week Sweden	Plasma cotinine >15.0 ng/mL = active smoking 0.1–15.0 ng/mL for exposure to SHS vs. <0.1 ng/mL No exposure to cigarette smoke	 2.1 (1.4–3.3) 1.7 (1.2–2.4)	Adjustment for age, country of birth, education, marital status, shift work, parity, previous SAB, caffeine intake, folate level, change of eating habits, pregnancy symptoms; SHS exposed may include light smokers
Nielsen et al. 2006	Nested case-control study from population-based cohort of 11,088 women with baseline survey and 2-year follow-up 343 SAB or fetal loss by 28 weeks' gestation, self-reported or in Danish registry 1,578 women with pregnancy of >28 weeks during follow-up period Denmark	Smoking at baseline, before pregnancy, by amount and number of years of smoking Amount (cigarettes/day) 1–14 15–19 ≥20 Each additional 5	1.0 (current smokers vs. never smoked) Reference group 1.4 (0.9–2.1) 1.6 (1.1–2.5) 1.2 (1.0–1.4)	Adjustment for age, marital status, previous SAB, oral contraceptive use, IUD use, and smoking status and duration of smoking—but did not affect ORs; no association for former smokers or by duration of smoking; odd reference group

Note: **CI** = confidence interval; **IUD** = intrauterine device; **ng/mL** = nanograms per milliliter; **OR** = odds ratio; **SES** = socioeconomic status; **SHS** = secondhand smoke.

smoking with ectopic pregnancy, and in a meta-analysis of data from nine studies, the OR from pooled data on ectopic pregnancy from smoking was 1.77 (95 percent CI, 1.31–2.22) (Castles et al. 1999). In addition, two other studies also reported significant associations between smoking and ectopic pregnancy (Bouyer et al. 2003; Karaer et al. 2006). Both found evidence of a dose-response relationship, even after adjustment for important potential confounders, such as a history of sexually transmitted diseases and infertility. In one study, the magnitude of the association of ectopic pregnancy and smoking was similar to that seen with infectious causes (Bouyer et al. 2003). In addition, plausible mechanisms for a relationship between smoking and ectopic pregnancy exist. The oviduct plays a critical role in the pickup and transport of the oocyte, and failure of this function can result in ectopic pregnancy. Both in vivo and in vitro studies showed that smoking impairs mammalian oviduct function (Talbot and Riveles 2005).

Preeclampsia

Preeclampsia is a syndrome of reduced organ perfusion attributable to vasospasm and endothelial activation with an onset after 20 weeks of gestation that is marked by proteinuria, hypertension, and dysfunction of the endothelial cells lining the uterus (National High Blood Pressure Education Program 2000; Sibai et al. 2005). The disease can be mild or severe. When accompanied by seizures that cannot be attributed to other causes, the diagnosis is established as eclampsia by exclusion. Preeclampsia affects approximately 2 to 8 percent of pregnancies (*American Journal of Obstetrics and Gynecology* 1988; Duley 2003; Zhang et al. 2003; Villar et al. 2004), and the incidence is highest in nulliparous women. The reported incidence varies widely, likely because of variation in population characteristics such as parity, race and/or ethnicity, and environmental factors (Zhang et al. 1997), as well as heterogeneity in classification systems (*American Journal of Obstetrics and Gynecology* 1988; Villar et al. 2004). Preeclampsia is a leading cause of pregnancy-related mortality in the United States (Berg et al. 2003). Morbidity and mortality are particularly high with early-onset disease (<33 weeks of gestation) (Sibai 2003; von Dadelszen et al. 2003). Preeclampsia is also associated with fetal growth restriction, placental abruption, and perinatal death (Sibai et al. 2005).

Risk factors for preeclampsia include preexisting medical conditions, multifetal gestation, an elevated body mass index (BMI), and older maternal age (Sibai et al. 2005). Immunologic factors have also been implicated (Zhang et al. 1997; Dekker and Robillard 2003; Einarsson et al. 2003), as have infectious and/or inflammatory conditions (Sibai et al. 2005). Evidence from epidemiologic and physiological studies has led to several hypotheses on the cause of preeclampsia. First, preeclampsia seems to be characterized by poor formation of the placenta (placentation) with a shallow invasion of the decidua and myometrium by trophoblast cells, resulting in an incomplete transformation of maternal spiral arteries that then retain their muscular characteristics (Brosens et al. 1972; Naicker et al. 2003). This process leads to placental ischemia and reperfusion and results in increased oxidative stress and vascular disease. Poor placentation in preeclamptic pregnancies could be the result of maternal-fetal immune maladaptation (Sibai et al. 2005). Researchers think that the clinical manifestations of preeclampsia result from the release of placental factors in response to ischemic conditions, resulting in the endothelial dysfunction of maternal circulation (Roberts and Redman 1993). Endothelial dysfunction is characterized by a disruption in regulatory functions of vasomotor tone through coagulation, by platelet activity, and by fibrinolysis in the vascular endothelium (Roberts et al. 1989, 1991). It is unclear which placental factors may be involved, but one hypothesis is that an imbalance between proangiogenic and antiangiogenic factors may contribute. Animal and human studies support the hypothesis that angiogenic proteins may play a role in the etiology of preeclampsia (Maynard et al. 2003; Levine et al. 2004).

Smoking is inversely associated with preeclampsia; the pooled risk reduction is 32 percent (Conde-Agudelo et al. 1999). The 2004 Surgeon General's report found the evidence sufficient to infer a causal relationship between smoking and a reduced risk of preeclampsia (USDHHS 2004). Whether a dose-response relationship exists is unclear because study results are conflicting (Marcoux et al. 1989; Klonoff-Cohen et al. 1993; Zhang et al. 1999). Investigators have proposed three mechanisms through which smoking could reduce the risk of preeclampsia (Maynard et al. 2003; Fisher 2004):

1. exposure to thiocyanate, which has a hypotensive effect (Andrews 1973);

2. inhibition of thromboxane A_2 production, a potent vasoconstrictor and platelet aggregation stimulator, or increase in levels of prostacyclin, a vasodilator and platelet aggregation inhibitor (Ylikorkala et al. 1985; Davis et al. 1987; Marcoux et al. 1989), both of which would improve the ratio of thromboxane A_2 to prostacyclin (Lindqvist and Maršál 1999); and

3. stimulation of proangiogenic factors, such as vascular endothelial growth factor (VEGF), and/or

reduction in antiangiogenic factors, such as soluble VEGF receptor Flt-1 (sFlt-1) (Maynard et al. 2003; Fisher 2004; Jeyabalan et al. 2008).

Placenta Previa

Placenta previa is the complete or partial obstruction of the cervical os by the placenta that affects approximately 0.4 percent of all births (Comeau et al. 1983; Iyasu et al. 1993; Faiz and Ananth 2003). Placenta previa has been associated with maternal and infant complications, such as preterm delivery, a hemorrhage that requires a blood transfusion, maternal death, and fetal or neonatal death (Salihu et al. 2003; Creasy et al. 2004). Neonatal mortality in pregnancies complicated by placenta previa may be up to three times higher than that in the general obstetric population (Salihu et al. 2003). The cause of placenta previa is unknown. However, risk factors with plausible etiologic mechanisms include advanced maternal age, multiparity, multifetal gestation, and a history of a cesarean section or a previous abortion (Ananth et al. 2003; Faiz and Ananth 2003; Creasy et al. 2004).

Epidemiologic studies have consistently reported an increased risk of placenta previa among smokers, and many studies show a dose-response relationship (Meyer et al. 1976; Zhang and Fried 1992; Monica and Lilja 1995). The estimated relative risks (RRs) from smoking are 1.3 to 3.0 (Castles et al. 1999; Andres and Day 2000; Cnattingius 2004). The 2004 Surgeon General's report found the evidence sufficient to infer a causal relationship between smoking and placenta previa (USDHHS 2004). A mechanism commonly proposed to explain this association is the chronic hypoxemia and ischemia that result from smoking, with compensatory placental enlargement. However, not all studies have shown a clinically significant increase in placental size in smokers (Zhang et al. 1999; Larsen et al. 2002).

Placental Abruption

Placental abruption, the premature separation of the placenta from the uterine wall, affects 0.5 to 2 percent of pregnancies (Rasmussen et al. 1996a; Ananth et al. 2001, 2005; Kyrklund-Blomberg et al. 2001). However, reported perinatal mortality in affected women is 8 to 12 percent (Raymond and Mills 1993; Ananth and Wilcox 2001; Kyrklund-Blomberg et al. 2001). Abruption may account for up to 14 percent of perinatal deaths (Rasmussen et al. 1996b; Ananth and Wilcox 2001). Ananth and Wilcox (2001) estimated that the perinatal mortality rate associated with abruption was 119 per 1,000 births compared with 8.2 per 1,000 among all births. Abruption can also result in neonatal asphyxia (Heinonen and Saarikoski 2001), preterm delivery, and maternal disseminated intravascular coagulation if thromboplastic material is released into the mother's circulatory system (Hladky et al. 2002).

It is likely that the etiology of placental abruption is multifactorial (Misra and Ananth 1999). Potential risk factors include advanced maternal age (35 years or older), high parity, previous abruption, a history of infertility, preterm premature rupture of membranes (PPROM), small for gestational age (SGA), infant congenital malformations, multifetal pregnancy, hypertensive disorders, polyhydramnios, thrombophilia, diabetes, trauma, sudden uterine decompression, previous cesarean section, and uterine infections (Abdella et al. 1984; Williams et al. 1991; Raymond and Mills 1993; Ananth et al. 1996a,b; Kramer et al. 1997; Rasmussen et al. 1999; Cunningham et al. 2001; Kyrklund-Blomberg et al. 2001). Underlying causes of abruption could include vessel fragility, vascular malformations, uterine scarring from previous cesarean section, and placentation abnormalities (Dommisse and Tiltman 1992; Rasmussen et al. 1999; Hladky et al. 2002). In addition, failure of the maternal spiral arteries to transform into low-resistance dilated vessels could predispose the mother to ischemia and vessel rupture (Eskes 1997). In one study, a high percentage of placentas from women with severe abruption showed an absence of trophoblastic transformation, and not all cases were attributable to preeclampsia (Dommisse and Tiltman 1992).

Studies have consistently associated smoking with an increased risk of placental abruption. Relative risks range from 1.4 to 1.9 (Raymond and Mills 1993; Ananth et al. 1999; Castles et al. 1999; Andres and Day 2000). The 2004 Surgeon General's report found the evidence sufficient to infer a causal relationship (USDHHS 2004). In addition, study findings support a dose-response relationship (Ananth et al. 1999). Raymond and Mills (1993) found a 20-percent increase in the risk of abruption for every 10 cigarettes the mother smoked per day. Etiologic mechanisms proposed by researchers to explain this relationship include smoking-related degenerative and/or inflammatory changes in the placenta (Cnattingius 2004); decreased vitamin C (ascorbic acid) levels in smokers (Faruque et al. 1995), leading to impaired collagen synthesis (Cnattingius 2004); microinfarcts; and atheromatous changes in placental vessels (Naeye 1979; Andres and Day 2000) (see "Placenta" and "Maternal and Fetal Cardiovascular System" later in this chapter). Analyses of consecutive pregnancies indicate that abruption risk is decreased when women stop smoking between pregnancies, suggesting that effects of smoking are transient and not cumulative across pregnancies (Ananth and Cnattingius 2007).

Preterm Delivery

Delivery at less than 37 completed weeks of gestation (preterm delivery) is a leading cause of neonatal mortality and morbidity in developed countries and is often divided into categories of moderate preterm (32 to 36 weeks) and very preterm (<32 weeks) delivery. Preterm delivery complicated 12.3 percent of pregnancies in the United States in 2003 (Hamilton et al. 2004). Rates of preterm delivery in the United States and other industrialized countries have been increasing in the past decade and are partially attributable to an increase in the frequency of multiple births (Hamilton et al. 2004).

The underlying causes of preterm delivery are complex and multifactorial. Contributing factors include multigestational pregnancy, preeclampsia, placental abruption, placenta previa, intrauterine infections, uterine overdistension, and abnormal uterine anatomy, in addition to disorders of the cervix, endocrine system, and placenta. Other risk factors include race (e.g., African Americans have higher risk), low socioeconomic status (SES), underlying maternal medical conditions, genitourinary infections, poor maternal weight gain or nutrition, young or advanced maternal age, short maternal stature, and fetal abnormalities (Haram et al. 2003; Iams 2003). Approximately 25 percent of preterm deliveries are medically indicated and are attributable to conditions affecting the mother and/or the fetus, and the remaining 75 percent are spontaneous (Meis et al. 1995; Iams 2003). A substantial body of evidence indicates that intrauterine bacterial infections are associated with preterm labor and delivery, especially at earlier gestational ages (Cassell et al. 1993; Kimberlin and Andrews 1998; Andrews et al. 2000; Goldenberg and Culhane 2003). Most intrauterine infections are believed to result from ascending infection, resulting from changes in vaginal/cervical flora, including bacterial vaginosis, or from the introduction of pathologic organisms. If these organisms ascend to the intrauterine cavity, they can cause an inflammatory reaction. The infection may progress to involve the chorion and/or amnion, fetal vessels, or the amniotic cavity, and even the fetus (review by Gonçalves et al. 2002; Romero et al. 2002). Systemic maternal infection or maternal infections remote from the genitourinary tract have also been associated with preterm labor and delivery, but the risk of preterm labor and delivery attributable to these conditions is thought to be low (Romero et al. 2002). One mechanism proposed to explain the onset of preterm labor attributable to intrauterine infection is that bacterial invasion of the choriodecidual space results in the release of endotoxins and exotoxins, which, in turn, stimulate the production of cytokines such as TNFα, interleukin-6 (IL-6), IL-8, IL-1α, IL-1β, and granulocyte colony-stimulating factor. One hypothesis is that cytokines, endoxins, and exotoxins stimulate the release of prostaglandins and initiate neutrophil activation, which results in the release of metalloproteases. This process results in stimulation of uterine contractions by prostaglandins, rupture of chorioamniotic membranes, and softening of the cervix by metalloproteinases (review by Goldenberg et al. 2000).

Researchers have consistently associated smoking with preterm delivery, and smoking likely increases the risk of both very preterm and moderate preterm births (Kyrklund-Blomberg and Cnattingius 1998; Ancel et al. 1999; Cnattingius et al. 1999; Gardosi and Francis 2000). The 2004 Surgeon General's report found that the evidence was sufficient to infer a causal relationship between smoking and preterm delivery (USDHHS 2004). Smoking appears to increase the risk of both medically indicated and spontaneous preterm delivery (Kyrklund-Blomberg and Cnattingius 1998). However, estimates of the magnitude of the association vary among studies. In a meta-analysis of pooled data from 20 prospective studies, the estimate for any maternal smoking versus none was 1.27 (95 percent CI, 1.21–1.33), and the ORs were 1.25, 1.38, and 1.31 for light, moderate, and heavy maternal smoking, respectively (Shah and Bracken 2000), suggesting a truncated dose-response relationship.

Exposure to secondhand smoke is also associated with preterm delivery in several studies. The 2006 Surgeon General's report concluded that the evidence was suggestive but not sufficient to infer a causal relationship (USDHHS 2006). A study by Kharrazi and colleagues (2004) included measurement of cotinine and found that nonsmokers with higher levels had earlier delivery than did those with no measurable exposure to secondhand smoke. The risk increased about 30 percent with each log increase in cotinine (adjusted OR [AOR] = 1.29 [95 percent CI, 0.97–1.72]).

Parity may modify the association between preterm delivery and smoking. As previously stated, the incidence of preeclampsia is highest among nulliparous women (see "Preeclampsia" earlier in this chapter). Because smoking protects against preeclampsia and preeclampsia can result in preterm delivery, the adverse effects of smoking on risk of preterm delivery may be masked in nulliparous women (Burguet et al. 2004).

Mechanisms through which smoking may contribute to preterm delivery are unknown. Researchers have proposed that smoking could increase risk of intrauterine infections (review by Cnattingius 2004). Smokers have a twofold-to-threefold increase in risk for bacterial vaginosis, which is a risk factor for preterm delivery (Morris et al. 2001). Researchers have hypothesized that smoking increases this risk through its effects on vaginal flora

or through depletion of Langerhans cells, resulting in local immunosuppression (Smart et al. 2004). Alterations in the cervical cytokine profile have been associated with increased risk of preterm delivery; women with a high anti-inflammatory and low proinflammatory profile are at highest risk (Simhan and Krohn 2009). Cigarette smoking has been associated with increased cervical anti-inflammatory cytokines in early pregnancy, which could make women who smoke more vulnerable to reproductive tract infections and subsequent preterm delivery (Simhan et al. 2005). Smoking can also reduce zinc levels, which could increase susceptibility to vaginal infections (Edman et al. 1986; Sikorski et al. 1990; Shubert et al. 1992). Immunosuppressive effects of smoking could also increase the risk of upper genital infections, known to be associated with preterm labor and PPROM. Neonates born to smokers have been noted to have a decrease in all leukocytes, indicating possible fetal immune dysregulation (Pachlopnik Schmid et al. 2007). Pathways other than those involving infections have also been proposed. For example, investigators have suggested that smoking during pregnancy increases contractile sensitivity and activity of the myometrium, with exposure to oxytocin by upregulating expression of messenger RNA (mRNA) for oxytocin receptor (Egawa et al. 2003). Compared with unexposed pregnant rats, those with exposure to cigarette smoke were found to have higher contractile sensitivity and activity in response to oxytocin (Egawa et al. 2003).

Finally, findings have also suggested that smoking may disrupt the integrity of type III collagen, leading to weakening and rupture of the membranes and an increased risk of medical indications for preterm delivery, such as placental abruption and intrauterine growth restriction (IUGR) (Cnattingius 2004).

Preterm Premature Rupture of Membranes

PPROM is defined as the rupture of amniotic membranes before the onset of labor and before 37 completed weeks of gestation. PPROM occurs in up to 4.5 percent of deliveries and in approximately 40 percent of preterm births (Mercer et al. 2000). Pregnancies complicated by PPROM have higher rates of neonatal morbidity than do pregnancies complicated by idiopathic preterm labor (Arias and Tomich 1982).

Factors that researchers have associated with PPROM include (1) nutritional deficiencies in vitamin C (Hadley et al. 1990; Casanueva et al. 1993), copper (Artal et al. 1979; Kiilholma et al. 1984), and zinc (Sikorski et al. 1990; Scholl et al. 1993); (2) vaginal bleeding (Harger et al. 1990; Ekwo et al. 1992); (3) multifetal pregnancies (Mercer et al. 1993); (4) a history of preterm delivery or PPROM in a previous pregnancy (Naeye 1982; Harger et al. 1990; Ekwo et al. 1992; Mercer et al. 2000); (5) obstetric complications involving uterine overdistension (French and McGregor 1996); and (6) bacterial vaginosis (Kurki et al. 1992; Mercer et al. 2000) and intra-amniotic infections (Naeye and Peters 1980; Ekwo et al. 1993; Heffner et al. 1993). However, it can be difficult to determine whether an intra-amniotic infection precedes or follows the rupture of membranes. A review by Lee and Silver (2001) discusses in detail the risk factors for PPROM.

Researchers suggest that structural deficiencies in the architecture of the amniotic membrane could increase the risk of PPROM (Shubert et al. 1992; Lee and Silver 2001). Studies of spontaneously ruptured membranes demonstrate that membranes are thinner and collagen content is lower near the site of rupture. Moreover, these alterations appear to be focal rather than generalized (Skinner et al. 1981; Kanayama et al. 1985; French and McGregor 1996). The tensile strength of tissue depends on the collagens, especially types I and III. The amniotic membranes of women with PPROM have decreased amounts of type III, type V, and total collagen (Skinner et al. 1981; Kanayama et al. 1985; al-Zaid et al. 1988). In addition, Athayde and colleagues (1998) found that women with PPROM had higher amniotic fluid levels of metalloproteinases that degrade collagen types IV and V than did women with term labor. These researchers also suggested that infection could be an additional trigger if the host responds to an infection by activating matrix-degrading enzymes (Athayde et al. 1998).

Many studies have associated smoking with an increased risk of PPROM (Lee and Silver 2001). The 2004 Surgeon General's report found sufficient evidence to infer a causal relationship between smoking and PPROM (USDHHS 2004). Researchers have hypothesized that smoking increases the risk of PPROM through several pathways. The effects of smoking on the immune system could increase the risk of genital tract infection or disrupt the cytokine system (French and McGregor 1996). Smoking could increase the inflammatory response and reduce the availability of nutrients such as vitamin C or decrease the uptake of nutrients by the placenta (French and McGregor 1996; Lykkesfeldt et al. 1996, 2000) (see "Other Molecular Mechanisms" later in this chapter).

Developmental Endpoints

This section on developmental effects summarizes the epidemiologic evidence for prenatal effects of maternal smoking on the infant and child, including endpoints such as birth weight, perinatal or infant mortality, birth defects, and neurobehavioral. The discussion also briefly

notes the evidence for effects from exposure to secondhand smoke on these outcomes. Studies have examined the effects of smoking on child growth, but this topic is not addressed in this report. Studying childhood health in relation to prenatal smoking is complicated by the possibility of exposure to secondhand smoke subsequent to birth, as well as other intervening factors between birth and some later outcomes that are difficult to assess.

Fetal Size and Growth

The first and the most widely studied effect of maternal smoking is the influence on fetal growth. Fetal growth cannot be directly assessed, so birth weight is used as a surrogate. However, birth weight reflects not only growth but also gestational age, as well as genetic potential, which is not commonly assessed. To account for gestational age, studies may examine IUGR, which is usually assessed from the distribution of birth weight by gestational week, in a standard population. The common definition of SGA is less than the 10th percentile of weight for the age. Another parameter that is examined includes low birth weight (LBW) (<2,500 grams [g]), sometimes among term births only (≥37 weeks).

The first Surgeon General's report on smoking and health in 1964 noted an association of maternal smoking with LBW (U.S. Department of Health, Education, and Welfare 1964). The 2001 Surgeon General's report on women and smoking concluded that "infants born to women who smoke during pregnancy have a lower average birth weight and are more likely to be SGA than are infants born to women who do not smoke" (USDHHS 2004, p. 15). Furthermore, the 2004 Surgeon General's report concluded that "the evidence is sufficient to infer a causal relationship between maternal active smoking and fetal growth restriction and low birth weight" (USDHHS 2004, p. 28). These conclusions are based on a multitude of studies with consistent evidence of a dose-response relationship, confirmed by more recent studies using a biomarker of exposure to tobacco smoke.

Infants of smokers typically weigh 150 to 200 g less than infants of nonsmokers and are twice as likely (ORs = 1.5 to 2.5) to be LBW or SGA. Maternal smoking appears to have the strongest effect on birth weight through growth retardation and, to a lesser extent, through a shortened gestation (Ananth and Platt 2004; USDHHS 2004). On the basis of a maternal smoking rate of about 12 percent during pregnancy, the etiologic fraction (EF) for LBW from smoking was calculated as 6.4 percent for all births and 10.9 percent for single births (Magee et al. 2004). The EF for LBW from smoking among births at full term was slightly higher at 13.4 percent or 16.7 percent for single births, which comprise most births. The authors also reported that 60 percent of the effect of smoking on LBW in the overall population was among light smokers.

Because of the established effects of maternal active smoking, many studies have also examined fetal growth in relation to exposure of the mother to secondhand smoke. Several reviews concluded that exposure to secondhand smoke is associated with adverse effects on infant growth or an increased risk of LBW and SGA (NCI 1999; WHO 1999a; USDHHS 2001; British Medical Association 2004). Moreover, the 2006 Surgeon General's report concludes that "the evidence is sufficient to infer a causal relationship between exposure to secondhand smoke and a small reduction in birth weight" (USDHHS 2006, p. 13). The highest quality studies indicate birth weight decrements of 15 to 100 g and an OR for LBW or SGA of 1.1 to 1.7 from exposure to secondhand smoke (NCI 1999). A meta-analysis of pooled data conducted through 1995 calculated a mean weight decrement of 28 g (95 percent CI, 16–40) among infants of mothers who did not smoke but were exposed to secondhand smoke and ORs of 1.2 for IUGR and 1.4 for LBW (Windham et al. 1999a). One study based on a sensitive assay for cotinine showed a birth weight decrement of 27.2 g (95 percent CI, 0.6–53.7) per unit change in log cotinine, which represented a decrement of about 100 g between the highest and lowest cotinine quintiles (Kharrazi et al. 2004).

Several studies have shown that the effects of exposure to tobacco smoke, primarily active but also involuntary exposure, appear to be stronger among older mothers (Wen et al. 1990; Wisborg et al. 1996; Haug et al. 2000; Windham et al. 2000; Salihu et al. 2005). Risk may also vary by racial and ethnic groups, and some studies (Mainous and Hueston 1994; USDHHS 1998; Windham et al. 2000) noted stronger effects among non-Whites or Blacks. Some of these variations may be a result of differences in nicotine metabolism among racial groups or differences in smoking and exposure patterns. Smoking may cause reduced birth weight or fetal growth due to fetal hypoxia resulting from exposure to CO, other effects on fetal nutrition, or the action of PAHs (see "Tobacco Smoke Toxicants and the Reproductive System" and "Other Molecular Mechanisms" later in this chapter).

Perinatal and Infant Mortality

The definition of perinatal mortality may vary slightly across studies, but it commonly includes stillbirth at more than 28 weeks of gestation and early neonatal deaths (first 7 days of life). Infant mortality includes death of a live-born child in the first year of life and can be divided into the neonatal (first month) and postneonatal (1 month to 1 year) periods. Neonatal mortality is more related to prenatal conditions. Previous reports of

the Surgeon General have reviewed the data for an effect of smoking on mortality. The 2001 report concluded, "the risk for perinatal mortality—both stillbirth and neonatal deaths—and the risk for sudden infant death syndrome (SIDS) are increased among the offspring of women who smoke during pregnancy" (USDHHS 2001, p. 15). The 2004 Surgeon General's report also concluded, "the evidence is sufficient to infer a causal relationship between sudden infant death syndrome and maternal smoking during and after pregnancy" (USDHHS 2004, p. 7). However, the report noted the difficulty of separating prenatal from postnatal effects of maternal smoking.

Many studies have found a slightly increased risk of approximately 20 to 30 percent for stillbirth or neonatal mortality associated with smoking (USDHHS 2004). A meta-analysis of pooled data from 23 cohort studies of perinatal mortality calculated a RR of 1.26 (95 percent CI, 1.19–1.34) (DiFranza and Lew 1995). In one study, the risk was higher for postneonatal death (AOR = 1.6; 95 percent CI, 1.41–1.85) than for neonatal death (AOR = 1.2; 95 percent CI, 1.05–1.30), which was primarily attributable to two causes: respiratory disease and SIDS (Malloy et al. 1988). A study in India (Gupta and Subramoney 2006) reports an adjusted risk for stillbirth three times higher for mothers who use smokeless tobacco than for those who do not use smokeless tobacco. Some evidence for a dose-response relationship was shown for frequency of use. Two previous studies from India had also reported an increased risk of stillbirth or perinatal death with use of smokeless tobacco (primarily chewing tobacco) (Krishna 1978; Shah et al. 2000). These investigations lend further support to the literature on the adverse effects of tobacco use, which may be related to heavy metals or nicotine, because CO from tobacco smoke would not be present (see "Tobacco Smoke Toxicants and the Reproductive System" later in this chapter).

Studies of SIDS and maternal smoking that controlled for other factors showed ORs of 1.8 to 3.1. Several of those studies also report a dose-response relationship (NCI 1999; USDHHS 2004). A meta-analysis comparing women who did or did not smoke during pregnancy, regardless of smoking status after delivery, calculated a pooled OR of 2.98 (95 percent CI, 2.51–3.54) (DiFranza and Lew 1995). Some studies have attempted to separate the effects of prenatal exposure from those of postnatal exposure. In one study, the risk of SIDS was increased in infants with only postpartum exposure to tobacco smoke but was even greater with both prenatal and postnatal exposures (Schoendorf and Kiely 1992). A large study in which participants were asked detailed questions on exposure to tobacco smoke found similar risks for maternal or paternal smoking, but the risks increased with more smokers in the household (Klonoff-Cohen et al. 1995). In addition, some studies that attempted to examine the independent effects of paternal smoking observed elevated risks of SIDS (Mitchell et al. 1993; Blair et al. 1996). Rates of SIDS have decreased over time with strong public education campaigns to place infants in the supine position while they sleep. Subsequent studies found that the risk attributable to maternal smoking has concomitantly increased and smoking may now be the greatest preventable cause of SIDS (Chong et al. 2004; Anderson et al. 2005).

Birth weight is one of the strongest predictors of infant survival. However, the effects of reduced growth versus shortened gestation are important to consider in determining etiology. Both reduced growth and shortened gestation appear to be related to infant mortality and SIDS (McCormick 1985; Oyen et al. 1995; Paneth 1995; Ananth and Platt 2004). The longer the gestation for a given birth weight, the lower is the mortality (McCormick 1985; Wilcox and Skjærven 1992). The increased risk of mortality associated with LBW appears to continue beyond infancy into childhood (Samuelson et al. 1998; Xu et al. 1998). Furthermore, studies suggest an association of maternal smoking with a higher mortality rate that continues beyond infancy. This effect was greater after adjustment for birth weight (Hofvendahl 1995). Infants who experience symmetrical growth retardation (in weight, length, and head circumference) associated with maternal smoking may be less likely to exhibit later "catch-up" growth and appear to be more likely to have cognitive deficits and difficulties in school (McCormick 1985). Thus, other effects associated with maternal smoking, such as perinatal mortality, may be mediated through reduced fetal growth and, to some extent, through a shortened gestation period (Ananth and Platt 2004). Some studies have found higher risks of infant mortality associated with smoking among mothers who are 35 years of age or older or of certain racial groups (Cnattingius et al. 1988; Li and Daling 1991). Studies have also found an increased risk of SIDS with placental abnormalities (Li and Wi 1999), thus suggesting another mechanism by which smoking may lead to SIDS.

Birth Defects

The 2004 Surgeon General's report summarized epidemiologic studies published between 1974 and 1998 on the relationship between maternal smoking during pregnancy and the risk for congenital malformations (USDHHS 2004). The report concluded that "the evidence is inadequate to infer the presence or absence of a causal relationship between maternal smoking and congenital malformations in general" (USDHHS 2004, p. 28). For oral clefts, however, several studies reported increased risks,

and the evidence was considered to be suggestive, but not sufficient, to infer a causal relationship with smoking.

Since the 2004 Surgeon General's report, additional studies have examined possible associations of maternal smoking with major birth malformations. The evidence in support of an association between smoking and an increased risk for oral clefts has become stronger (Table 8.3). A meta-analysis of data from studies published between 1974 and 2001 focused on 9 studies that examined total orofacial clefts and 15 that examined cleft lip with or without cleft palate and cleft palate alone. The study found an association between maternal smoking and a 34-percent increase in the risk of cleft lip with or without cleft palate and a 22-percent increase in the risk of cleft palate alone (Little et al. 2004a). More recently, similar findings were also observed by Honein and colleagues (2007). Other studies provide evidence of a dose-response relationship for maternal smoking and the risk for cleft lip with or without cleft palate (Wyszynski and Wu 2002; Little et al. 2004b; Honein et al. 2007) and for cleft palate alone (Little et al. 2004b). A recent study assessing maternal tobacco exposure by cotinine levels in mid-pregnancy serum samples found an OR of 2 for cleft lip with or without cleft palate (Shaw et al. 2009).

Studies on interactions between genes and smoking and between vitamin use and smoking contribute to an understanding of the etiology of oral clefts (van Rooij et al. 2002; Jugessur et al. 2003; Shi et al. 2007) (see "Fetal Tissue and Organogenesis" and "Smoking and Maternal and Neonatal Genetic Polymorphisms" later in this chapter). One study reported an increase in the risk for cleft lip with or without cleft palate that was strongest among offspring whose mothers had smoked but had not consumed multivitamins during the periconceptive period (Shaw et al. 2002). Results did not suggest an interaction for isolated cleft palate. Study findings also supported an increased risk of clefting with paternal smoking or involuntary exposure to tobacco smoke (Savitz et al. 1991; Zhang et al. 1992; Shaw et al. 1996). Whether paternal smoking acts through exposure of the mother to secondhand smoke or directly on male gametes is not clear.

A number of studies have also investigated maternal smoking in relation to cardiovascular malformations. A review of data from 13 studies published between 1971 and 1999 found mixed results. Twelve of the studies provided results for cardiovascular malformations combined and seven studies for specific subgroups of cardiovascular defects examined separately (Källén 2002a). Combining all cardiovascular malformations represents a heterogeneous group but examining subgroups resulted in low power for several studies. The conflicting results probably reflect differences in research methods, including case ascertainment, classification, control of confounding, and sample size of the case group. Other studies have reported associations of maternal smoking with cardiovascular malformations (Table 8.4), including conotruncal defects, atrial septal defects, and atrioventricular septal defects (Torfs and Christianson 1999); L-transposition of the great arteries (Steinberger et al. 2002; Kuehl and Loffredo 2003); and conotruncal defects among offspring whose mothers did not use vitamins (Shaw et al. 2002). Steinberger and colleagues (2002) found an association between paternal smoking and single-ventricle defects. These more recent exploratory studies present methodologic issues similar to those noted in the meta-analysis by Källén (2002a). In addition, Malik and colleagues (2008) found an association between periconceptual smoking and septal heart defects. There is a need for further research with large, population-based studies that incorporate standardized methods for case ascertainment and classification to determine whether a relationship exists between maternal smoking and the risk of cardiovascular malformations.

Studies of other birth defects have found an association between maternal smoking and an increased risk for clubfoot (Honein et al. 2000; Skelly et al. 2002), craniosynostosis (Källén 1999; Carmichael et al. 2008), and gastroschisis (Werler et al. 2003), but not for spina bifida (Table 8.5) (Shaw et al. 2002; van Rooij et al. 2002). Studies report mixed results for maternal smoking and limb deficiency defects (Hwang et al. 1998; Shaw et al. 2002; Carmichael et al. 2004), Down syndrome (Chen et al. 1999; Yang et al. 1999), and for cryptorchidism and hypospadias (Akre et al. 1999; Källén 2002b; Pierik et al. 2004).

Maternal smoking may interfere with normal organ development in offspring in several ways, including through fetal hypoxia, alterations in essential nutrients, teratogenic effects, and DNA damage. Those effects may be related to exposure to tobacco smoke components such as CO, nicotine, cadmium, and PAHs (Chernoff 1973; Mochizuki et al. 1984; Lammer et al. 2004; Munger et al. 2004; Ziaei et al. 2005). In addition, certain populations with genetic polymorphisms may be more susceptible to damage attributable to exposure to tobacco smoke because of alterations in metabolic pathways (see "Fetal Tissue and Organogenesis" and "Smoking and Maternal and Neonatal Genetic Polymorphisms" later in this chapter).

Neurodevelopment

Maternal smoking and exposure to secondhand tobacco smoke during pregnancy affect infant health status at birth as described earlier and are hypothesized to affect physical and mental development in infancy and early childhood as well. Studies have reported evidence of lower weights and shorter heights into the preschool period (Fox et al. 1990), in addition to correlations of

maternal smoking with microcephaly and hydrocephaly, particularly among female infants (Honein et al. 2001). Reviews have also examined the links between maternal smoking and mental development in offspring (Hardy and Mellits 1972; Lassen and Oei 1998). Earlier Surgeon General's reports examined this topic and reported possible effects. However, at the time of the 2004 Surgeon General's report, the evidence was considered "inadequate to infer the presence or absence of a causal relationship between maternal smoking and physical growth and neurocognitive development of children" (USDHHS 2004, p. 601), and this conclusion was echoed in the examination of secondhand smoke (USDHHS 2006). Some key studies, as well as others published since those included in the prior reports of the Surgeon General (e.g., 2004 and later), are summarized below.

Researchers have suggested that prenatal exposure to smoking poses a unique risk for neurologic development and intellectual abilities attributable to impairments of the central nervous system (Olds et al. 1994). Drews and colleagues (1996) studied a sample of 221 children aged 10 years and reported that those with mental retardation were more likely than control participants with no mental retardation to have mothers who had smoked during pregnancy. Moreover, the rates of retardation increased with the number of cigarettes mothers smoked. McCartney and colleagues (1994) speculated that intrauterine exposure to nicotine specifically affects the physiology of outer hair cells in the ear that underlies language ability and leads to poorer performance scores among offspring on assessments that rely heavily on verbal abilities.

Investigators have found it difficult to document a consistent impact of maternal smoking on cognitive development in infants and young children, because many factors affect cognitive development. For example, in a study of two- and four-year-old children of mothers who smoked compared with children in the same age groups whose mothers did not smoke, Baghurst and colleagues (1992) reported small but significant group differences on the Bayley Scales of Infant Development (BSID) and the McCarthy Scales of Children's Abilities (MSCA). However, group differences were not significant after controlling for SES, maternal intelligence quotient, and quality of the home learning environment. Sexton and colleagues (1990) also reported better scores among three-year-olds whose mothers had stopped smoking during pregnancy compared with children whose mothers had smoked more than 10 cigarettes a day during pregnancy. Group differences in performance on the Minnesota Child Development Inventory and the MSCA were small, but differences remained significant after adjustment for birth weight, SES, and certain maternal and child characteristics. Trasti and colleagues (1999) reported lower scores on the Wechsler Preschool and Primary Scale of Intelligence-Revised for a sample of 369 children aged five years whose mothers had smoked during pregnancy compared with children of mothers who did not smoke. However, significant group differences were not found after adjustment for maternal education level. These researchers also reported no differences on the BSID in a sample of 376 children from the same population at 13 months of age by mothers' smoking status.

Batstra and colleagues (2003) reported poorer performance on mathematics and spelling achievement tests among a group of 1,186 children aged 5 through 11 years whose mothers smoked, and differences remained after adjustment for SES and for prenatal and perinatal complications. A Danish study found effects of prenatal smoking during the third trimester on adult intelligence even after adjustment for sociodemographic variables (Mortensen et al. 2005). In contrast to other studies, Eskenazi and Trupin (1995) reported slightly higher but nonsignificant scores on the Peabody Picture Vocabulary Test and the Raven Progressive Matrices Test for five-year-old children whose mothers had smoked during pregnancy compared with those for children of mothers who had not smoked, even after adjustment for parental education, SES, age, race, and preschool attendance. Some significant decrements in performance on these same measures and significant differences in the maternal-rated activity levels were attributable to exposure to secondhand tobacco smoke during childhood. Other studies show cognitive deficits with prenatal exposure to secondhand smoke that are exacerbated by conditions of material hardship (Rauh et al. 2004). After adjustment, decrements in cognitive and academic abilities were reported with increasing cotinine levels within the range indicating exposure to secondhand smoke during childhood (Yolton et al. 2005).

Despite these inconsistent findings on general assessments of children's cognition and intelligence, findings more consistently show an association between maternal smoking and children's lower performance on assessments of verbal skills in general, as well as on specific language and auditory tests. For example, a sample of 110 children aged 6 to 11 years whose mothers had smoked during pregnancy performed more poorly on tasks tapping phonologic processing skills that are known to be related to both language and reading abilities (McCartney et al. 1994). Follow-up studies of the same cohort reported that maternal smoking and maternal involuntary exposure to tobacco smoke negatively affected the performance of children aged 9 and 12 years on standardized assessments of language and reading, as well as on assessments of general intelligence skills (Fried et al. 1997). Butler and Goldstein (1973) studied a sample of more than 9,000 children aged 7 and 11 years whose

Table 8.3 Association between maternal smoking and orofacial clefts (OFCs), 1999–2009[a]

Study	Design/population	Definition of smoking (smoking rate in controls)
van Rooij et al. 2001	Case-control study 113 infants with nonsyndromic OFCs 104 infants with no defects White, aged 9 months–3 years The Netherlands Study period not specified	Smokers (20.2%) vs. nonsmokers
Shaw et al. 2002	Case-control study 489 infants and fetal deaths with OFCs 734 control infants with no malformation California Birth Defects Monitoring Program 1987–1989	Smoking in periconceptional period—1 month before to 3 months after conception—(24%) vs. none
van Rooij et al. 2002	Case-control study 45 mothers of children with OFCs 39 mothers of children with spina bifida 75 control mothers DNA for possible effect modification The Netherlands 1997–1999	Smokers (18.1%) vs. nonsmokers Smoking in periconceptional period Number of cigarettes/day
Wyszynski and Wu 2002	Case-control study 2,029 births with nonsyndromic oral clefts 4,050 control infants with no malformation, matched on maternal and paternal race and child's gender, county of birth, and month of birth United States Natality database 1997	Smoking Any (16.9%) vs. none Number of cigarettes/day 1–10 (10.8%) 11–20 (4.9%) >20 (1.1%)
Jugessur et al. 2003	Case-parent triad study 261 case-parent triads (88 CPO and 173 CL/P) Norway 1996–1998	Smoking in last year or during first trimester Before pregnancy (31%) First trimester (27%)
Little et al. 2004a	Meta-analysis of data from 24 case-control and cohort studies 9 studies addressed only OFCs 15 studies assessed CL/P and CP separately Studies published between 1974 and 2001	Smokers vs. nonsmokers varied by study

Findings	OR (95% CI)	Comments
• Maternal smoking not associated with oral clefting	1.1 (0.6–2.2)	See Table 8.13 for polymorphism results
• Association of maternal smoking with isolated CL/P among women not using vitamins Vitamins/Smoking Yes/No No/Yes No/No Yes/Yes • Relationship marginally significant for isolated CP Vitamins/Smoking Yes/No No/Yes No/No Yes/Yes	 1.0 2.8 (1.8–4.3) 1.9 (1.3–2.7) 1.5 (1.0–2.2) 1.0 2.0 (1.0–3.9) 1.6 (1.0–2.6) 1.7 (1.0–2.9)	Adjustment for maternal body mass index, education, race, and ethnicity
• Association with OFCs • No association between maternal NAT2 acetylator status and OFCs • No interaction between NAT2 acetylator status and maternal smoking	2.0 (0.8–5.0)	Adjustment for maternal education
• Maternal smoking associated with CL/P in infants Any vs. none • Number of cigarettes/day vs. none 1–10 11–20 >20	 1.16 (1.01–1.33) 1.10 (0.93–1.30) 1.11 (0.87–1.41) 1.55 (0.88–2.70) Trend test, p = 0.072	Did not distinguish cases with isolated CP; adjusted for maternal education, age, race, diabetes mellitus, and pregnancy-associated hypertension
• Risk for smoking alone not stated • No interaction between maternal smoking and *TGF*α gene *TAQ1 *A2/*A2* genotype in infant		None
• Consistent, moderate, and statistically significant associations between maternal smoking and CL/P • Evidence of a dose-response relationship between maternal smoking and CP	1.34 (1.25–1.44) 1.22 (1.10–1.35)	None

Table 8.3 Continued

Study	Design/population	Definition of smoking (smoking rate in controls)
Little et al. 2004b	Case-control study 190 children with OFCs 248 children with no malformation Scotland and Manchester and Merseyside regions of England 1997–2000	Number of cigarettes/day Active smoking and involuntary exposure to cigarette smoke Maternal smoking Ever (37.5%) During pregnancy (24.2%) First trimester (23.8%) Exposure to cigarette smoke among mothers who did not smoke (28.2%) Exposure to cigarette smoke among mothers who smoked (31.0%)
Honein et al. 2007	Case-control study 933 CL/P 528 CPO 3,390 controls with no defects 8 sites in the United States Oct 1997–Dec 2001 births	Periconceptual smoking (1 month before to 3 months after conception) 20% controls exposed
Shi et al. 2007	Case-control study DNA samples from 1,244 children with CL/P, CPO 4,183 parents, siblings, and unrelated population controls Danish samples from two independent case-control studies (Dec 1991–Aug 1994; born between 1981–1990) Iowa samples from cases in Iowa Registry for Congenital and Inherited Disorders Control children born without major anomalies were matched by birth month, year, and gender	Number of cigarettes/day 0 1–9 10–19 ≥20
Shaw et al. 2009	Case-control study nested within a cohort of pregnancies with mid-pregnancy serum specimens 89 pregnancies with CL/P 409 pregnancies without malformations	Mid-pregnancy serum cotinine levels No smoke exposure: cotinine value <2 µg/mL Any exposure: cotinine value ≥2 µg/mL

Note: **CI** = confidence interval; **CL/P** = cleft lip with or without cleft palate; **CP** = cleft palate; **CPO** = cleft palate only; **mL** = milliliter; **OR** = odds ratio; **µg** = micrograms.
[a]Not included in 2004 Surgeon General's report on the health consequences of smoking.

Findings	OR (95% CI)	Comments
• First trimester smoking associated with CL/P		Adjustment for gender, season of birth, maternal education, and infant's ethnic group
Any vs. none	1.9 (1.1–3.1)	
≤10 cigarettes/day	1.7 (0.9–3.0)	
>10 cigarettes/day	2.5 (1.1–5.6)	
• First trimester smoking associated with CP		
Any vs. none	2.5 (1.3–4.1)	
≤10 cigarettes/day	2.1 (1.1–3.9)	
>10 cigarettes/day	3.1 (1.2–7.8)	
• An effect of involuntary smoking could not be excluded in mothers who did not smoke		
• CL/P	1.3 (1.0–1.6)	Strong effects for bilateral CL/P and heavy smoking
• CPO	1.2 (0.9–1.5)	
• Maternal smoking was associated with OFC in both Danish and Iowa data		Suggestive effects of variants in the *NAT2* and *CYP1A1* genes were observed in both the Iowan and Danish participants
CL/P		
Danish		
10–19	1.71 (1.14–2.56)	
≥20	1.16 (0.51–2.64)	
Iowa		
1–4	0.93 (0.42–2.57)	
5–14	2.24 (1.11–4.50)	
≥15	2.78 (1.42–5.44)	
CPO		
Danish		
10–19	1.17 (0.61–2.23)	
≥20	0.35 (0.05–2.67)	
Iowa		
1–4	0.91 (0.30–2.75)	
5–14	2.52 (1.09–5.81)	
≥15	1.58 (0.59–4.21)	
• There was an interaction between maternal smoking and fetal inheritance of a *GSTT1 null* deletion (Fisher combined p value <0.001)		
• Association of maternal serum mid-pregnancy cotinine levels with CL/P	2.1 (1.0–4.4)	Exposure assessment based on biomarker

Table 8.4 Association between maternal smoking and cardiovascular malformations, by phenotype, 1999–2008

Study	Design/population	Definition of smoking (prevalence of smoking)
Torfs and Christiansen 1999	Case-control study 687 infants with Down syndrome 385 case infants with cardiac defects also 302 control infants with no heart defects and no other specific defect California Birth Defects Monitoring Program 1991 and 1993	First trimester participants Yes vs. no Smoking during pregnancy 12% of all participants 8% of controls for cardiac defects
Woods and Raju 2001	Retrospective cohort study of 18,016 live births in TriHealth Hospital System (Cincinnati, Ohio) Congenital defects grouped in 22 categories, including oral clefts 1987–1999	Smokers vs. nonsmokers Cohort included 1,943 mothers who were smokers
Källén 2002a	Meta-analysis of 13 case-control studies published in 1971–1999	Smokers vs. nonsmokers None, 1–20 cigarettes/day, and >20 cigarettes/day Prevalence varied by study
Shaw et al. 2002	Case-control study 207 infants and fetal deaths with conotruncal defects 489 infants and fetal deaths with orofacial clefts 265 infants and fetal deaths with neural tube defects 734 control infants with no malformation California Birth Defects Monitoring Program 1987–1989	Maternal smoking during periconceptional period (1 month before to 3 months after conception) Smoking among case mothers Smoking among controls
Steinberger et al. 2002	Case-control study 48 infants with single ventricle and abnormal situs 3,572 infants without cardiac defects Baltimore-Washington Infant Study (Maryland; northern Virginia; and Washington, DC) 1981–1989	Parental smoking of >1 pack/day (maternal 4.9%, paternal 9%) vs. no smoking Exposure period during gestation not stated
Kuehl and Loffredo 2003	Case-control study 36 infants with single L-transposition of great arteries 3,495 infants without cardiac defects Baltimore-Washington Infant Study Maryland; northern Virginia; and Washington, DC) 1981–1989	Not stated Prevalence of maternal smoking for control infants not mentioned
Malik et al. 2008	Case-control study 3,067 infants with congenital heart defects 3,947 controls without defects	Those who reported smoking any time from 1 month before conception and through each month of pregnancy <1 cigarette/day to >2 packs/day

Findings by type of cardiovascular defect	Odds ratio (95% confidence interval)	Comments
• Maternal smoking associated with grouped cardiac defects • Maternal smoking associated with Atrioventricular septal defects Tetralogy of Fallot Atrial septal defects	2.1 (1.2–3.5) 2.3 (1.2–4.5) 4.6 (1.2–17.0) 2.2 (1.1–4.3)	Adjustment for maternal race; all infants had Down syndrome
• Smoking associated with increased risk of cardiovascular system abnormalities • Patent ductus arteriosus (n = 153) and ventricular septal defect (n = 48) most common abnormalities • Maternal smoking not associated with 21 other defects	1.56 (1.12–1.82)	Adjustment for maternal age, race, and diabetes mellitus but not for gestational age
• Mixed results for cardiovascular defects overall • Mixed results for specific cardiovascular defects	Data not reported	Different methods of case ascertainment, classification, control of confounding, and small case groups
• Smoking associated with conotruncal defects among infants of women who did not use vitamins Vitamins/Smoking Yes/No No/Yes No/No Yes/Yes	 1.0 2.2 (1.3–4.0) 1.4 (0.9–2.2) 1.0 (0.6–1.7)	Adjustment for maternal body mass index, education, race, and ethnicity
• No association with maternal smoking • Increased risk with paternal smoking of >1 pack/day • Increased risk with paternal smoking of increasing number of cigarettes/day 1–19 20–39 ≥40	1.9 (0.6–6.3) 2.2 (1.1–5.1) 1.0 1.9 3.7 trend test, p = 0.02	Source of information on paternal smoking not specified; small case sample size; estimates based on exact methods but not adjusted for potential confounders
• Maternal cigarette smoking associated with increased risk of L-transposition of great arteries	1.6 (1.1–2.4)	Maternal smoking highly correlated with use of hair dyes; adjustment for maternal use of hair dyes, paternal exposure to laboratory chemicals, and residence in spatial cluster
• Septal heart defects Light smoking Medium smoking Heavy smoking	 1.44 (1.18–1.76) 1.50 (1.11–2.03) 2.06 (1.20–3.54)	None

Table 8.5 Association between maternal smoking and noncardiovascular congenital malformations, by type of malformation, 1998–2008

Study	Design/population	Analysis and definition of smoking (smoking rate in controls)
Hwang et al. 1998	Case-control study 34 infants with isolated LDD 482 control infants with other isolated defects Maryland Birth Defects Reporting and Information System 1984–1992	Maternal smoking during pregnancy (27.5% among all participants) Any vs. none Effect modification by *MSX1* gene
Reefhuis et al. 1998	Case-control study 3,662 children with foot deformities 7,829 control children with chromosomal or monogenic disorder 26 European Surveillance of Congenital Anomalies registries 1980–1994	During pregnancy Any (18.1%) vs. none
Akre et al. 1999	2 population-based case-control studies 2,782 boys operated on for cryptorchidism 1,220 boys operated on for hypospadias (5 matched controls per case) Linkage of Inpatient and Birth Registries Sweden birth cohort 1983–1993	Prenatal clinic record on maternal smoking Yes (38.1%) vs. no
Chen et al. 1999	Case-control study 775 children with Down syndrome 7,750 control children with no malformation, matched by birth year Washington State Birth Events Records Database 1984–1994	Any maternal smoking during pregnancy vs. none (age) <35 years (20.2%) ≥35 years (14.2%)
Källén 1999	Case-control studies 304 infants with craniosynostosis, no information available on type or number of controls Linkages of Swedish Registry of Congenital Malformations and National Board of Health Medical Birth Registry Sweden 1983–1996	Smoking at first prenatal visit Any (% not stated) vs. none Categories None <10 cigarettes/day ≥10 cigarettes/day

Findings by type of defect	Odds ratio (95% confidence interval)	Comments
• Interaction with *MSX1 *rare* alleles and risk of isolated limb deficiency		Passive surveillance system, so incomplete case ascertainment; small sample; unadjusted
Smoking only	0.7 (0.03–6.72)	
1 or 2 alleles only	2.4 (0.73–8.71)	
Smoking and 1 or 2 alleles	4.8 (1.37–18.4)	
• Maternal smoking during pregnancy associated with increased risk of foot deformities	1.2 (1.1–1.3)	Adjustment for maternal age, registry, parity, and year of birth; control infants with malformation
• Maternal smoking associated with increased risk of cryptorchidism	1.19 (1.06–1.33)	Adjustment for maternal age, parity, height, history of subfertility, preeclampsia, twinning, Apgar score, other malformations, gestation, and birth weight
• Maternal smoking not associated with increased risk of hypospadias	0.85 (0.71–1.02)	
• Maternal smoking during pregnancy not associated with Down syndrome	1.00 (0.82–1.24)	Adjustment for exact year of maternal age, race, and parity
• Associated with craniosynostosis	1.45 (1.13–1.87)	Adjustment for year of birth, maternal age, parity, and educational level; no information on smoking prevalence
• Associated with isolated craniosynostosis	1.67 (1.27–2.19)	
• Not associated with syndromes	0.61 (0.29–1.30)	
• Dose-response effect for isolated craniosynostosis		
<10 cigarettes/day	1.48 (1.04–2.02)	
≥10 cigarettes/day	2.12 (1.50–2.99)	
• Associated with sagittal but not other suture synostosis	1.48 (1.02–2.14)	

Table 8.5 Continued

Study	Design/population	Analysis and definition of smoking (smoking rate in controls)
Yang et al. 1999	Population-based case-control study 285 children with Down syndrome 329 control children with no defects Cases ascertained from Metropolitan Atlanta Congenital Defects Program 5 counties in Georgia 1989–1996	Lifetime nonsmokers (71.0%) Ever smoked (28.9%) Current smoker (15.8%) Total pack-years[a] <5 (16.4%) ≥5 (12.2%) Effect modification by timing and origin of meiotic error and OC use
Honein et al. 2000	Population-based case-control study 346 infants with isolated clubfoot 3,029 infants with no defects Frequency matched to cases by year and hospital of birth Atlanta Birth Defects Case-Control Study 1968–1980	First trimester Any (28.6%) vs. none
Källén 2002b	Case-control study 3,262 infants with hypospadias 1,413,811 infants with no malformation Linkages of Swedish Registry of Congenital Malformations, National Board of Health Medical Birth Registry, and Hospital Discharge Registry Sweden 1983–1996	Smokers (24.6%) vs. nonsmokers Cigarettes/day <10 (15.2%) ≥10 (9.3%)
Shaw et al. 2002	Case-control study 265 infants and fetal deaths with neural tube defects 165 infants with LDD 734 control infants with no malformation California Birth Defects Monitoring Program 1987–1989	During the periconceptional period (1 month before to 3 months after conception) (24%) vs. none

Findings by type of defect	Odds ratio (95% confidence interval)	Comments
• No association in presence of maternal meiosis I nondisjunction error		60–63% participation rates; interaction of smoking with type of nondisjunction error and OC use
Current smoker (age)		
<35 years	0.69 (0.35–1.37)	
≥35 years	0.81 (0.20–3.29)	
• Associated with Down syndrome in young mothers with meiosis II nondisjunction error		
Ever smoked (age)		
<35 years	2.43 (0.89–6.63)	
≥35 years	0.37 (0.07–1.87)	
Current smoker (age)		
<35 years	2.98 (1.01–8.87)	
≥35 years	0.65 (0.06–7.48)	
• Associated with Down syndrome in young mothers with OC use and maternal meiosis II nondisjunction error		
OC use alone	0.38 (0.05–3.01)	
Smoking without OC use	1.54 (0.45–5.27)	
Smoking with OC use	7.92 (1.63–35.6)	
• Interaction between maternal smoking and family history of clubfoot		Adjustment for gravidity and gender
Smoking only	1.32 (1.04–1.72)	
Family history only	6.52 (2.95–14.41)	
Both	20.30 (7.90–52.17)	
• Maternal smoking negatively associated with hypospadias	0.83 (0.76–0.90)	Stratification by maternal age, parity, educational level, and year of birth
Smokers vs. nonsmokers		
<10 cigarettes/day	0.84 (0.76–0.93)	
≥10 cigarettes/day	0.81 (0.71–0.92)	
• Little association with LDD		Adjustment for maternal BMI, education, race, and ethnicity
Vitamins/Smoking		
Yes/No	1.0	
No/Yes	1.5 (0.8–2.8)	
No/No	1.1 (0.7–1.8)	
Yes/Yes	0.8 (0.5–1.4)	

Table 8.5 Continued

Study	Design/population	Analysis and definition of smoking (smoking rate in controls)
Skelly et al. 2002	Case-control study 239 children with clubfoot 365 control children with no malformation Western Washington State 1986–1994	Average number of cigarettes/day during pregnancy None (81.7%) 0.01–9.9 (11.2%) 10–19.9 (4.8%) ≥20 (2.3%)
van Rooij et al 2002	Case-control study 45 case mothers of children with orofacial clefts 39 case mothers of children with spina bifida 75 control mothers The Netherlands 1997–1999	Smokers (18.1%) vs. nonsmokers Cigarettes/day Effect modification by NAT2 acetylator status
Werler et al. 2003	Population-based case-control study Mothers of 205 case children with gastroschisis 127 case children with small intestinal atresia 381 malformed control children 416 control children with no malformation 15 cities across the United States and Canada 1995–1999	Maternal smoking during first trimester Cigarettes/day Control children with malformation Any (34%) 1–9 (10%) 10–19 (11%) >19 (13%) Control children with no malformation Any (34%) 1–9 (9%) 10–19 (9%) >19 (16%)
Carmichael et al. 2004	Population-based case-control study 92 infants with LDD 180 control infants with no malformation California Birth Defects Monitoring Program 1987–1988	Maternal and paternal smoking and infant *MSX1* genotype Neither (50.0%) Mother only (11.7%) Father only (20.6%) Both (14.4%)
Pierik et al. 2004	Population-based nested case-control study 78 case children with cryptorchidism 56 case newborns with hypospadias 313 control newborns with no defects in a cohort of 8,698 male newborns Rotterdam, The Netherlands 1999–2001	Current smoking (maternal 22.7%, paternal 41.5%) vs. no current smoking

Findings by type of defect	Odds ratio (95% confidence interval)	Comments
• Associated with idiopathic clubfoot		No confounders noted or adjusted for; no information collected on vitamin supplement intake
Smoking vs. none (cigarettes/day)	2.1 (1.5–3.3)	
0.01–9.9	1.5 (0.9–2.5)	
10–19.9	3.1 (1.7–5.8)	
≥20	3.9 (1.6–9.2)	
Risk (any vs. none) varied by infant gender		
Boys	1.8 (1.2–3.0)	
Girls	2.8 (1.4–5.4)	
• Associated with isolated idiopathic clubfoot		
Any vs. none (cigarettes/day)	2.4 (1.7–3.6)	
0.01–9.9	1.4 (0.9–2.5)	
10–19.9	3.5 (1.8–6.7)	
≥20	4.6 (1.9–11.0)	
• Maternal smoking not associated with spina bifida • No association between maternal NAT2 acetylator status and spina bifida • No interaction	Data not reported	Adjustment for maternal education
• Associated with gastroschisis		Adjustment for vasoconstrictive drug use and maternal age
Any vs. none (cigarettes/day)	1.5 (1.1–2.2)	
1–9	1.3 (0.7–2.2)	
10–19	1.4 (0.9–2.4)	
>19	1.8 (1.1–2.8)	
• Not associated with small intestinal atresia		Adjustment for age, education, family income, and use of vasoconstrictive drugs, other medications, alcohol, and marijuana
Any vs. none (cigarettes/day)	1.0 (1.6–1.7)	
1–9	1.4 (0.6–1.7)	
10–19	0.8 (0.3–2.0)	
>19	1.2 (0.6–2.4)	
• No association of maternal smoking and LDD risk	1.0 (0.4–2.3)	None
• No association of paternal smoking and LDD risk	1.1 (0.6–2.2)	
• No association between *MSX1* genotype and LDD • No interaction between parental smoking, infant's *MSX1* genotype, and LDD risk		
• Maternal smoking not associated with cryptorchidism	1.3 (0.8–2.3)	Adjustment for small-for-gestational-age status, self-reported exposure to solvents, and time to pregnancy
• Maternal smoking not associated with hypospadias	1.6 (0.8–2.3)	
• Paternal smoking not associated with cryptorchidism	1.2 (0.6–2.1)	
• Paternal smoking associated with hypospadias	3.8 (1.8–8.2)	

Table 8.5 Continued

Study	Design/population	Analysis and definition of smoking (smoking rate in controls)
Carmichael et al. 2008	Case-control study 531 cases of craniosynostosis 5,008 control children without malformation matched by birth cohort National Birth Defects Prevention Study 10 states 1997–2003	Smoking before pregnancy, 1st, 2nd, and 3rd month of pregnancy 2nd and 3rd trimesters Categories None Smoked <5 cigarettes/day Smoked 5–14 cigarettes/day Smoked ≥15 cigarettes/day

Note: **Apgar** = appearance, pulse, grimace, activity, respiration; **BMI** = body mass index; **LDD** = limb deficiency defects; **OC** = oral contraceptive.
[a]Pack-years = the number of years of smoking multiplied by the number of packs of cigarettes smoked per day.

mothers had smoked during pregnancy. These children were three to five months behind children of nonsmoking mothers in reading, mathematics, and general cognitive abilities. In more than 5,000 youth aged 6 through 16 years from the Third National Health and Nutrition Examination Survey, deficits in reading and mathematics scores were associated with higher current exposure to secondhand smoke (Yolton et al. 2005).

Researchers have used various types of tests that measure both cognitive and behavioral aspects of development to study the relationship between possible language impairments and maternal smoking. Data from studies using evoked brain responses indicate that infants born to mothers who smoked approximately one pack of cigarettes per day showed atypical patterns of brain organization, which reflected poorer speech discrimination than that of infants born to mothers who did not smoke. Compared with infants of smokers, the unexposed infants exhibited more common forms of brain lateralization for speech and showed evidence of better discrimination of consonant and vowel syllables (Molfese et al. 2004). This finding parallels findings in studies that reported long-term impacts on language and cognitive domains in children whose mothers smoked (Makin et al. 1991; McCartney et al. 1994). These results indicate that prenatal exposure to smoking in otherwise healthy infants can be linked to significant changes in brain physiology associated with basic perceptual skills. These effects may be long term, with impacts noted in later school performance. A study of gravid mice exposed to tobacco smoke supports these findings. The study revealed that the offspring had a developmental delay in neonatal reflexes and notable behavioral deficits in adulthood, including impaired learning and memory abilities (Li and Wang 2004). CO in tobacco smoke induces fetal hypoxia and may contribute to these effects (see "Carbon Monoxide" later in this chapter).

Findings by type of defect	Odds ratio (95% confidence interval)	Comments
• Smoking during first month of pregnancy was not associated with craniosynostosis		Adjustment for maternal age, education, race/ethnicity, subfertility, parity, folic acid supplement intake, BMI, and study center
• Smoking later in pregnancy (2nd or 3rd trimester) was associated with increased risk but only among mothers who smoked at least 1 pack per day		
• Smoked ≥15 cigarettes/day		
2nd trimester	1.6 (0.9–2.8)	
3rd trimester	1.4 (0.7–2.5)	
• Most confidence intervals include 1.0		

Pathophysiological and Cellular and Molecular Mechanisms of Smoking

This section explores various mechanisms by which smoking may affect reproductive and developmental outcomes at the pathophysiological and cellular levels.

Endocrine System

One mechanism by which smoking may contribute to various reproductive outcomes is alterations in hormone function. Researchers have suggested that smoking has antiestrogenic effects (Baron et al. 1990). However, there is also evidence of effects on hormones other than estrogen, which may vary by gender and the stage of life.

Premenopausal Women

Hormone function has been difficult to study in non-clinic-based populations, because of the cyclic nature of hormone excretion and day-to-day variations in premenopausal women. During regular menstrual cycles, the hormone dynamics are predictable in a pattern that reflects the integrity of the hypothalamic-pituitary-gonadal (HPG) axis. Excretion of the follicle-stimulating hormone (FSH) from the pituitary gland is critical for ovarian follicle recruitment, development, and maturation (van Santbrink et al. 1995). The synthesis and excretion of estrogen by the follicles reflect ovarian activity that then modulates the release of gonadotropins from the pituitary through a negative-feedback loop. After ovulation has occurred, the follicle undergoes luteinization, and the corpus luteum excretes progesterone and some estrogen to prepare the uterine lining for implantation. In the absence of conception, estrogen and progesterone levels decline, followed by menstruation. Should fertilization occur, the steroid levels continue to rise along with levels of other hormones, such as human chorionic gonadotropin (hCG), to maintain the pregnancy. The placenta takes over hormone production during pregnancy.

Smoking has been considered potentially antiestrogenic (Baron et al. 1990), primarily because of the nature of its association with hormonally related diseases such as reproductive cancers. However, the 2001 Surgeon General's report on women and smoking concluded that circulting levels of the major endogenous estrogens are not altered among smokers (USDHHS 2001). Some studies were hampered by a lack of biosampling points, small numbers of participants, or the inclusion of postmenopausal or potentially perimenopausal women. Details on 15 studies of premenopausal women are provided in Table 8.6. Most of these studies excluded women who were taking hormones or who were known to have menstrual problems. Nevertheless, the studies represent a variety of ages and did not always adjust for factors such as age and obesity. Two studies (MacMahon et al. 1982b; Westhoff et al. 1996) reported levels of urinary excretion of estrone, estradiol, and/or estriol in the luteal phase among smokers that were lower than those among nonsmokers, suggesting reduced estrogen production. However, several other studies did not observe significant differences in serum levels of estradiol by smoking status among premenopausal women (Longcope and Johnston 1988; Key et al. 1991; Berta et al. 1992). The study by Michnovicz and colleagues (1986) is often cited for the discovery that smoking induced the 2-hydroxylation of estrone to relatively inactive metabolites and decreased estriol excretion. A later, much larger study, however, did not find differences in the circulating levels of the 2α- or 16α-hydroxy metabolites in nulliparous smokers versus nonsmokers after adjustment for age, ethnicity, and length of menstrual cycle (Jernström et al. 2003). Zumoff and colleagues (1990) measured serum at multiple points during the cycle and found that estradiol was actually increased among heavy smokers in the follicular phase, particularly early, at baseline. A study with only a single serum sample obtained in the early follicular phase (Lucero et al. 2001) found that current smokers had higher baseline estradiol levels than did former olifetime nonsmokers, but this finding was not significant after adjustment. Examination of dose by pack-years[1] did not indicate a dose-response pattern, but this analysis may have been diluted by including former smokers with smokers if the effects do not persist after smoking cessation.

Among women who had IVF, smokers had higher baseline levels of 17-β-estradiol than did nonsmokers (Weigert et al. 1999). A later study examined hormonal dynamics by daily measurement of urinary levels of estrone and progesterone metabolites throughout the cycle, in relation to smoking level that was verified by cotinine bioassay (Windham et al. 2005). This analysis showed that heavy smokers had elevated baseline (e.g., early follicular phase) levels of the steroid metabolites, a finding consistent with results in other studies (Longcope and Johnston 1988; Zumoff et al. 1990; Key et al. 1996; Lucero et al. 2001). A study of Chinese nonsmokers examined the effects of secondhand smoke on urinary levels of hormone metabolites and found an association with lower mean levels of estrone conjugates only for nonconceptive cycles (Chen et al. 2005).

Some of the disease patterns observed with smoking may reflect changes in androgen or progesterone levels, rather than estrogen levels, or changes in the ratio of androgens to estrogens. Some studies have reported that smoking increases adrenal activity and have found elevations in adrenal androgens among postmenopausal smokers (Friedman et al. 1987; Khaw et al. 1988; Longcope and Johnston 1988; Key et al. 1991). Researchers have found elevated serum testosterone levels in female smokers that were positively correlated with estradiol levels in menstrual cycling women who had IVF (pre-hCG treatment) (Barbieri et al. 2005). Elevated testosterone levels in female smokers were also positively correlated with obesity (Longcope and Johnston 1988; Sowers et al. 2001). Zumoff and colleagues (1990) reported elevated serum levels of progesterone among heavy smokers during the early follicular phase, a time when most progesterone is from the adrenal cortex. This finding is again consistent with the urine metabolite results reported by Windham and colleagues (2005) (Table 8.6). However, Zumoff and colleagues (1990) observed little difference in progesterone levels in the luteal phase. Windham and colleagues (2005) observed dampened progesterone metabolites in the luteal phase with heavy smoking. Berta and colleagues (1992) found that regular smokers had lower plasma levels of progesterone in a single sample per day during the mid-luteal phase. However, the small study by Westhoff and colleagues (1996) did not find these differences in examining data on all smokers without considering the amount smoked. In addition, the study on exposure to secondhand smoke did not report reductions in progesterone metabolite levels with exposure (Chen et al. 2005).

In vitro experiments support the effects on progesterone by showing that granulosa and tumor cells treated with alkaloids found in cigarette smoke or with an aqueous extract of cigarette smoke showed a dose-dependent inhibition of progesterone production (Bódis et al. 1997; Gocze et al. 1999; Gocze and Freeman 2000; Miceli et al. 2005). In contrast, estradiol production was little affected or was slightly stimulated. Cell growth and DNA content

[1]Pack-years = the number of years of smoking multiplied by the number of packs of cigarettes smoked per day.

also decreased with treatment, leading the authors to suggest that smoking directly inhibits cellular progesterone synthesis through less specific cytotoxic effects on progesterone-producing cells (Gocze and Freeman 2000). Other proposed mechanisms include modulations in the prostaglandin system (Miceli et al. 2005) or inhibition of aromatase enzymes.

Some studies examined gonadotropin FSH levels by smoking status (Table 8.6). Three studies that measured a single serum FSH level in the first few days of the cycle found higher levels associated with smoking (Cramer et al. 1994; Cooper et al. 1995; Lucero et al. 2001). Another study with a similar finding of higher FSH in smokers did not include the time during the menstrual cycle when the single serum sample was obtained (Backer et al. 1999). In addition to the limitation of single serum samples, these studies tended to include some perimenopausal or postmenopausal older women even though FSH levels naturally rise during and after menopause. Another study measured daily urinary levels of FSH metabolites in women of reproductive age. These findings also showed mean FSH levels among moderate-to-heavy smokers (≥10 cigarettes per day) that were higher than those among nonsmokers during the luteal-follicular phase transition between cycles (Windham et al. 2005).

Serum levels of FSH increase with age, and researchers think this increase reflects the diminishing supply of oocyte- and gonadotropin-responsive follicles that leads to the release of the HPG axis from ovarian control (Marcus et al. 1993; Cramer et al. 1994; Westhoff et al. 1996). The FSH level is thus considered a marker of ovarian reserve or competence, and as such, it may also be useful for identifying agents with toxic effects on the ovaries (Scott et al. 1989; Scott and Hofmann 1995). As progesterone modulates FSH in the endocrine feedback loop, the lower levels of luteal phase progesterone metabolites observed in some studies are consistent with the decreased entrainment of FSH, which would lead to the observed elevations. The increase in FSH may accelerate the recruitment and development of follicles, moving ovulation earlier, and perhaps leading to inadequate follicle development followed by inadequate function of the corpus luteum. Progesterone controls the endometrial response and is critical for early maintenance of pregnancy. Studies have implicated a luteal phase deficiency as a cause of infertility and fetal loss (Pittaway et al. 1983; Tulppala et al. 1991). Earlier ovulation would also be consistent with the shortening of the menstrual cycle or of the follicular phase observed in smokers (see "Menstrual Function, Menopause, and Menarche" earlier in this chapter). The pattern of higher FSH levels, shorter cycles, and thus more frequent ovulation in smokers is also consistent with the observation that smokers tend to experience earlier menopause (see "Menstrual Function, Menopause, and Menarche" earlier in this chapter).

Pregnant Women

The 2001 Surgeon General's report noted that smoking more clearly affects estrogen levels during pregnancy than when a woman is not pregnant (USDHHS 2001). Several studies show that smokers have lower circulating levels of estriol and estradiol than do nonsmokers (USDHHS 2001), confirmed for estriol measured multiple times throughout pregnancy (Kaijser et al. 2000). Furthermore, the study found a positive correlation of estriol and birth weight. This study's results support the hypothesis of Michnovicz and colleagues (1986) that smokers and nonsmokers may metabolize estrogens differently, with acceleration of the 2-hydroxylation versus the 16α-hydroxylation pathway in smokers. In addition, some studies show an increase in 2-hydroxylation and 4-hydroxylation activity in placental tissues of smokers (Chao et al. 1981; Juchau et al. 1982). In a later study using placental microsomes, smokers had increased placental formation of 4-hydroxyestradiol, 7α-hydroxyestradiol, and most markedly, 15α-hydroxyestradiol, but little or no difference in the overall rate of placental estradiol metabolism or in the formation of the estrone, 2α-hydroxyestradiol, and other metabolites (Zhu et al. 2002). A study of progesterone in placental tissue samples revealed that levels among smokers were lower than those among nonsmokers (Piasek et al. 2001), a finding consistent with the data for nonpregnant women.

Using stored serum samples, Kandel and Udry (1999) found that the cotinine levels were positively correlated with the testosterone levels, especially in samples obtained during the second trimester of pregnancy. In turn, maternal testosterone levels were correlated with those in adult daughters. An animal study also showed that nicotine infusion resulted in increased plasma testosterone in ovine fetuses. This study also associated maternal exposure to nicotine with increased testosterone levels in 30-day-old (adolescent) female offspring of rats but not in male offspring (Smith et al. 2003). Changes in hormone patterns during pregnancy may therefore affect both pregnancy outcome and the endocrine profile of the offspring, thus relating to possible effects on neurobehavioral endpoints, puberty, or later reproductive status, including semen quality.

Men

Numerous studies have also examined hormone levels in men in relation to smoking (Table 8.7). Some studies examined the acute effects of smoking cigarettes in a standardized protocol, and others studied baseline

Table 8.6 Association between smoking and reproductive hormones in women

Study	Population	Sample/period	Definition of smoking (number of smokers)
MacMahon et al. 1982b	106 White women Aged 25–49 years	Overnight urine sample FP (days 10–11) LP (days 21–22)	Nonsmokers: ≤100 cigarettes in lifetime Former smokers (n = 23) Current smokers (n = 39)
Michnovicz et al. 1986	27 nonobese women with normal cycle length Mean age ~30 years	Blood and urine samples over 48 hours after administration of radiolabeled estradiol FP (days 1–10) in morning 5 samples in LP (days 19–25)	Smokers (14) ≥15 cigarettes/day
Longcope and Johnston 1988	88 women (47 premenopausal) Mean age 50 years	Radiolabeled steroid infusion Blood and urine samples at baseline	Smokers (23) 10 cigarettes/day for ≥10 years
Zumoff et al. 1990	16 volunteers with normal ovulatory and luteal function Aged 25–35 years	Blood samples on 17 days over cycle, more frequently around mid-cycle (ovulation)	Smokers (8) ≥20 cigarettes/day for ≥3 years
Key et al. 1991	147 women with cycle lengths of 21–35 days tested for estradiol and progesterone Aged ≥35 years Guernsey Breast Cancer Study 105 women tested for urinary steroids	24-hour urine and 1 blood sample	Current smokers (1–10, 11–20, ≥21 cigarettes/day) 69 smokers tested for estradiol and progesterone 20 smokers tested for urinary steroids
Berta et al. 1992	684 nonobese fertile women Aged 25–52 years	24-hour urine sample and 3 blood samples on days 21–24 of LP	Smokers (237) >10 cigarettes/day for ≥5 years
Cramer et al. 1994	224 women Aged 26–50 years Some with family history of ovarian cancer	1 blood sample in early FP (days 1–3)	Not defined, except "current, past, none" Current smokers (42)
Cooper et al. 1995	290 women Aged 38–49 years	1 blood sample in early FP (days 2–4)	Self-reports on current smoking (31), former smoking, and involuntary and prenatal exposure

Findings in smokers	Estimate of effect (95% CI or p value)	Comments
• Estrone, estradiol, and estriol levels in FP similar to those in nonsmokers • All 3 decreased in LP • Little difference from levels in former smokers	LP decreased 29% (10–43%)	Stronger effects in ovulatory cycles; adjustment for age; data on progesterone not presented
• 2-hydroxylation increased • Urine estriol and estriol/estrone decreased	Increased 50%, p <0.001 Decreased 40%, p <0.01	No difference in FP vs. LP; age and BMI similar in nonsmokers and smokers, so no adjustments made
• Metabolic clearance rates for testosterone, androstenedione, and estrone decreased • Plasma testosterone, androstenedione increased • No difference in estrone and estradiol or conversion from androgens	p <0.05 for metabolic clearance rates NS in only premenopausal women	No difference with adjustment for weight
• Progesterone increased in FP, not LP • Estradiol increased in early FP, slightly through FP • Mean LH slightly decreased	Increased 37% (p <0.0001) Increased 23% (p <0.001) in early FP Increased 12.5% (p ~0.05) through FP NS	Suggests that smoking stimulates adrenocorticol hormone secretion
• No difference in serum estradiol or progesterone • No difference in 6 urinary steroids (primarily androgens)	NS	Adjustment of serum values for cycle day, age, and BMI; adjustment of urine values for age, BMI, and parity
• Plasma progesterone decreased • Plasma prolactin decreased • No difference in estrone, estradiol, FSH, and LH	Decreased 15% (p <0.05) Decreased 20% (p <0.002)	Results not affected by age
• Mean FSH increased in current and former smokers (≥10 pack-years)[a]	Increased 14–21% (p = 0.03)	Effect of smoking still significant after adjustment for age
• Mean FSH increased in current smokers and those exposed to SHS • No difference in FSH in former smokers or prenatal exposure	Increased 66% (28–116%) Increased 57% at <10 cigarettes/day Increased 76% at 10–20 cigarettes/day Increased 39% (4–86%) with involuntary exposure	None of factors examined were confounders; significant interaction with age

Table 8.6 Continued

Study	Population	Sample/period	Definition of smoking (number of smokers)
Key et al. 1996	167 women Aged ≥34 years Guernsey Breast Cancer Study	24-hour urine sample with timing known and periovulatory period excluded	Self-reports, not defined (53)
Westhoff et al. 1996	175 parous women with normal cycles Aged 21–36 years	Urine sample on day 10 to menses Blood sample 3 times in LP	Self-reports, not defined (48)
Backer et al. 1999	3,114 women Aged 35–60 years National Health and Nutrition Examination Survey (U.S. national sample)	1 convenient blood sample	Smoking during 5 days before examination
Lucero et al. 2001	498 women not in depressive state Aged 36–44 years Harvard Study of Moods and Cycles	1 blood sample in early FP (days 1–5)	Smoking not defined; current smokers (52); former smokers (172)
Sowers et al. 2001	511 White women Aged 24–45 years	1 blood sample in FP (days 3–7) annually for 3 years	Lifetime nonsmokers, former smokers, current smokers (~20%)
Jernström et al. 2003	513 nulliparous women Aged 17–35 years	Morning blood sample on random day (timing calculated)	Current smoker (yes/no)
Windham et al. 2005	403 women in reproductive years Aged 18–39 years Members of Kaiser Permanente health maintenance organization	Daily urine collection through 2–7 cycles Adjustment of hormone values for creatinine	Cigarettes/day from daily diary, averaged over cycle Validated by cotinine 100–150 cycles of smokers

Note: **BMI** = body mass index; **CI** = confidence interval; **FP** = follicular phase; **FSH** = follicle-stimulating hormone; **LH** = luteinizing hormone; **LP** = luteal phase; **NS** = not statistically significant; **SHS** = secondhand smoke.
[a]Pack-years = the number of years of smoking multiplied by the number of packs of cigarettes smoked per day.

hormone levels in smokers compared with those in nonsmokers. The results from studies spanning many years are inconsistent. These studies also vary in considering obesity, which may be important because increased weight is associated with the peripheral conversion of androgens to estrogens. Also, studies generally report total circulating levels of hormones but vary in reports of free or bioavailable levels.

The most consistent finding is an increase of androstenedione in smokers in three studies (Barrett-Connor and Khaw 1987; Dai et al. 1988; Field et al. 1994). Testosterone levels also increased with smoking in many studies, but some studies found decreases or no differences (Table 8.7). The study by Sofikitis and colleagues (1995) is noteworthy for demonstrating differences between apparent endocrine versus paracrine levels of testosterone related to effects from smoking. Animal studies show that prenatal exposure to nicotine is related to decreased testosterone levels in adult male rats (Segarra and Strand 1989)

Findings in smokers	Estimate of effect (95% CI or p value)	Comments
• Estrone and estradiol slightly decreased • Estriol decreased in early FP, but less so later	0–16% for estrone 4–15% for estradiol 30% for estriol in early FP 5–22% for estriol later in cycle	NS after adjustment for time of cycle, age, and BMI
• No difference in mean LP progesterone in blood or urine • Urine estradiol decreased in LP	 21% decreased, p = 0.04	4 anovulatory women excluded; adjustment for creatinine (decreased differences in estradiol); some dose-response effects
• FSH increased	p <0.001	Cycle timing unknown, but tried to exclude samples obtained close to time of ovulation on basis of LH to FSH ratio
• FSH increased • Estradiol increased • LH increased • No difference in sex hormone binding globulin	12% increased, p <0.05 8% increased, p <0.05 42% increased, NS	No difference with former smoking; estradiol NS in adjusted model, but FSH values not adjusted; adjustment for age, BMI, calories, use of alcohol and caffeine, cholesterol, and cycle day
• Increased serum testosterone • Intermediate effect among former smokers	30–40% increased (by year), p <0.01	Significant after adjustment for BMI, year, and reproductive status
• 2α- and 16α-hydroxyestrone slightly increased before adjustment, but not after adjustment • No effect on either metabolite alone	19% increased, p = 0.06 unadjusted, p = 0.76 adjusted	Adjustment for age, race, and cycle day
• Mean FSH increased in smokers ≥10 cigarettes/day • Estrone and progesterone metabolites increased in early FP in smokers of ≥10 cigarettes/day • Progesterone decreased in LP in smokers of ≥20 cigarettes/day	20–35% increased, significant on some days 25% and 35% increased, p <0.05 25% decreased, p = 0.06	Adjustment for age, race, pregnancy history, BMI, and use of alcohol and caffeine; some dose-response effects

and that cotinine, but not nicotine, inhibits testosterone synthesis in testes of neonatal rats (Sarasin et al. 2003).

Four studies that measured estradiol or estrone had inconsistent results. However, results from one of the studies showing an association with smoking were not adjusted for BMI or weight, although adjustment was made for age. The findings for the gonadotropins, FSH and luteinizing hormone (LH), tend to show no effect of smoking.

In 1990, one study (Barrett-Connor 1990) suggested that the ratio of androgen to estrogen is critical in determining the gender-specific risk of some hormone-related diseases and that smoking may alter this ratio. Nicotine or its metabolites may influence endocrine profiles directly and indirectly. PAHs also act on the cytochrome P-450 systems involved in the metabolism of endogenous hormones and of xenobiotics such as those found in tobacco smoke.

Table 8.7 Association between smoking and reproductive hormones in healthy men

Study	Population	Study period	Definition of smoking
Briggs 1973	6 healthy smokers 6 healthy nonsmokers	7 days	>30 cigarettes/day
Dotson et al. 1975	91 university students attending simulated party	4 hours	Number of cigarettes smoked
Winternitz and Quillen 1977	17 long-term male smokers	2 days smoking followed by day not smoking	8 cigarettes 2.8 mg of nicotine within 2 hours vs. baseline on day of abstinence
Shaarawy and Mahmoud 1982	25 healthy smokers 20 healthy nonsmokers All fathers of ≥2 children	3 consecutive days	>20 cigarettes/day
Wilkins et al. 1982	10 long-term male smokers (≥1 pack/day of cigarettes with 1.0 mg of nicotine)	2 days	2 cigarettes 2.0 mg vs. 0.2 mg of nicotine in 10 minutes
Deslypere and Vermeulen 1984	75 healthy smokers 73 healthy nonsmokers 33 former smokers	Single blood sample	>5 cigarettes/day for >2 years
Klaiber et al. 1984	Group I 23 healthy smokers 18 healthy nonsmokers Group II 18 smokers 17 nonsmokers Attending infertility clinic	Single fasting blood sample for estradiol	Not defined
Gossain et al. 1986	6 healthy smokers 6 healthy nonsmokers	Experimental session with blood sample obtained 120 minutes after smoking first cigarette	2 unfiltered cigarettes smoked in 10 minutes
Seyler et al. 1986	4 healthy smokers Average 22.5 cigarettes/day	2 experimental sessions	15 hours with no smoking followed by 2 low-dose (0.48 mg) cigarettes smoked 5 minutes apart or 2 high-dose (2.87 mg) nicotine cigarettes smoked in rapid succession

Findings	Odds ratio/response rate and 95% CI or p value	Comments
• Serum testosterone lower in smokers (5.15 ± 0.7 ng/mL) than in nonsmokers (7.47 ± 0.53 ng/mL) • Mean increase in testosterone after 7 days abstinence for smokers (1.65 + 0.5 ng/mL) but no change for nonsmokers	NR p <0.005	No men obese
• Testosterone positively correlated with number of cigarettes smoked, r = 0.24	p <0.05	Study designed to look at testosterone levels, aggressive behavior, alcohol use, and smoking
• LH, FSH, testosterone, and thyroid-stimulating hormone showed no significant change during 4-hour study	NS	None
• Smokers had higher serum FSH (18.3 vs. 12.2 milli-international units per milliliter) • Serum testosterone was lower in smokers than in nonsmokers (3.6 vs. 6.2 ng/mL) • LH not different between groups	p <0.0025 p <0.0005	None
• Prolactin concentrations were significantly higher with high- vs. low-nicotine cigarettes • 150% increase in prolactin concentrations 30 minutes after smoking cigarette with 2 mg of nicotine and remained above baseline at 60 minutes	p <0.0001	None
• Plasma testosterone and free testosterone levels were higher in smokers than in nonsmokers for three age groups • LH did not differ between smokers and nonsmokers	Aged 20–39 years, p <0.001 Aged 40–59 years, p <0.01 Aged 60–80 years, p <0.05	Decrease in difference in plasma levels of testosterone and free testosterone in smokers vs. nonsmokers with increasing age
• Higher mean serum estradiol levels in both groups of smokers • Number of cigarettes smoked/day not associated with estradiol concentrations in smokers • Positive correlation with number of cigarettes smoked/day in analysis of pooled data from smokers and nonsmokers	Group I: p <0.03 Group II: p <0.001 Group I: r = 0.35, p <0.03 Group II: r = 0.73, p <0.001	Adjustment for age and alcohol intake
• Mean prolactin concentrations of 8.3 ng/mL for smokers and 15.4 ng/mL for nonsmokers	Integrated curves for 2 groups differed at p <0.001	None
• No differences in FSH, LH, prolactin, or thyroid-stimulating hormone concentrations between baseline and either high-dose or low-dose exposures	NS	None

Table 8.7 Continued

Study	Population	Study period	Definition of smoking
Barrett-Connor and Khaw 1987	590 men No history of heart disease or stroke Rancho Bernardo, California	Enrollment 1972–1974	Lifetime nonsmokers, former smokers, or current smokers (number of cigarettes/day)
Dai et al. 1988	121 men Pittsburgh Multiple Risk Factor Intervention Trial	Baseline and 4 years later	Cigarettes/day None 1–30 31–70
Meikle et al. 1989	75 healthy monozygotic twins 85 healthy dizygotic twins 1 set of healthy triplets		Nonsmokers, former smokers, current smokers Packs/day for current smokers
Michnovicz et al. 1989	20 healthy smokers 16 healthy nonsmokers	48 hours	Lifetime nonsmokers vs. ≥15 cigarettes/day
Field et al. 1994	1,241 healthy men Boston area 341 male smokers	Enrollment 1987–1989	Cigarettes smoked/day
Sofikitis et al. 1995	Healthy men presenting for hernia repair 49 smokers 18 lifetime nonsmokers 9 former smokers	19 months Presurgery and postsurgery sampling and postcessation sampling	≥20 cigarettes/day for >3 years
English et al. 2001	25 smokers 25 lifetime nonsmokers	NR	Self-reported ≥10 cigarettes/day
Trummer et al. 2002	260 smokers 70 former smokers 258 lifetime nonsmokers Infertility clinic population	1993–2000	Self-reported number of cigarettes/day

Note: **BMI** = body mass index; **CI** = confidence interval; **DHT** = dihydrotestosterone; **FSH** = follicle-stimulating hormone; **LH** = luteinizing hormone; **mg** = milligrams; **ng/mL** = nanograms per milliliter; **NR** = not reported; **NS** = no significant difference; **SHBG** = sex hormone binding globulin.

Findings	Odds ratio/response rate and 95% CI or p value	Comments
• No significant differences between groups in concentrations of testosterone or SHBG • Androstenedione was higher in smokers • Estrone was higher in smokers • Estradiol was higher in smokers • Dose-response effect for number of cigarettes/day for androstenedione, estrone, and estradiol, but not for testosterone and SHBG	 p <0.001 p <0.001 p <0.02 p <0.001	Adjustment for age, BMI, alcohol and caffeine intake, and exercise
• Testosterone positively associated with number of cigarettes/day • Androstenedione positively associated with number of cigarettes/day	p <0.05 p <0.05	Adjustment for age, alcohol use, high-density lipoprotein cholesterol, blood pressure, and relative weight
• Higher DHT concentrations for current smokers than for nonsmokers • No adjusted, independent effect of smoking for estrone, estradiol, testosterone, SHBG, LH, and FSH	p = 0.03	Study of effects of smoking on concentrations of sex steroid hormone through BMI
• Estradiol 2-hydroxylation increased ~70% in smokers • Urinary catechol estrogen index significantly higher for smokers	p <0.001 p = 0.006	None
• Higher androstenedione, testosterone, DHT, and SHBG for smokers	Androstenedione p = 0.0001 Testosterone p = 0.009 DHT p = 0.004 SHBG p = 0.0004	Adjustment for age and BMI
• Testicular androgen-binding protein lower in smokers • Testicular venous testosterone lower in smokers • Circulating serum FSH, LH, and testosterone concentrations not statistically different between smokers and nonsmokers	p <0.05 p <0.05	Included examination of testicular biopsy specimens
• Cotinine correlated with SHBG (r = 0.49) • Free testosterone and total testosterone higher in smokers • Bioavailable testosterone did not differ between groups • 17-β estradiol did not differ between groups	p <0.05 p = 0.03 p = 0.01	Matched on age and BMI
• Increased LH and testosterone and decreased prolactin in smokers compared with those for nonsmokers	Increased total and free testosterone, p <0.001 Increased LH, p = 0.035 Decreased prolactin, p <0.001	Testosterone lower in men who were able to stop smoking

Tubal Function

The mammalian oviduct transports gametes to the fertilization site and provides a suitable environment for fertilization and development before implantation. Factors that impair oviductal physiology can lead to reproductive problems, such as fertilization failure, ectopic pregnancy, and failure of implantation. Numerous epidemiologic studies have correlated maternal smoking with reproductive problems that can originate in the oviduct, including increased infertility and ectopic pregnancy (Stillman et al. 1986; Buck et al. 1997) (see "Review of Epidemiologic Literature on Smoking" earlier in this chapter).

The mammalian oviduct has three anatomic regions: (1) the infundibulum, which picks up the oocyte cumulus complex after it is ovulated from the ovary; (2) the ampulla, where fertilization occurs; and (3) the isthmus, which conducts sperm to the ampulla and provides a site for preimplantation development. Proper functioning of each region is necessary for normal reproduction.

The oviduct is an in vivo target of cigarette smoke and its components. Contraction of both the human oviduct (Neri and Eckerling 1969) and the rabbit oviduct (Ruckebusch 1975) is altered by exposure to tobacco smoke. Inhalation of mainstream or sidestream smoke at doses that produce serum cotinine levels within the range of those found in active smokers and persons involuntarily exposed to tobacco smoke caused blebbing of the oviductal epithelium and decreased the ratio of ciliated to secretory cells in hamsters (Magers et al. 1995). In another study of hamsters, in which the oviduct was directly observed before, during, and after inhalation of tobacco smoke at doses equivalent to those received by humans, both mainstream and sidestream smoke decreased ampullary smooth muscle contractions and slowed embryo transport through the oviduct (DiCarlantonio and Talbot 1999). Nicotine altered the motility of oviducts of rhesus monkeys (Neri and Marcus 1972), decreased oviductal blood flow (Mitchell and Hammer 1985), decreased sodium and potassium levels in oviductal epithelial cells of mice (Jin et al. 1998), and increased lactate dehydrogenase levels in oviductal epithelium of rats (Rice and Yoshinaga 1980).

In vitro studies using oviductal explants have been valuable in characterizing the effects of cigarette smoke on various biologic processes, including ciliary beat frequency, oocyte pickup rate, and smooth muscle contraction (Huang et al. 1997; Riveles et al. 2003). Solutions of particulate matter, whole mainstream smoke, and mainstream and sidestream smoke in the gas phase inhibited ciliary beat frequency, oocyte pickup rate, and smooth muscle contraction in a dose-dependent manner in hamsters (Knoll and Talbot 1998). Although this inhibition was originally reported for unfiltered (2R1) research cigarettes, a similar inhibition was subsequently shown for filter-tipped (1R4F) research cigarettes, unfiltered and filter-tipped commercial cigarettes, and "harm-reduction" cigarettes that are lower in carcinogens than are traditional brands (Riveles 2004). The data on harm-reduction cigarettes are important in demonstrating that these cigarettes still contain toxicants that can adversely affect diverse biologic processes. Exposure to sidestream whole smoke, in contrast to mainstream smoke, stimulated ciliary beat frequency (Knoll and Talbot 1998). The oocyte pickup rate was inhibited even in samples in which beat frequency was stimulated, which shows that pickup depends on factors other than ciliary beating. Adhesion between the extracellular matrix of the oocyte cumulus complex and the tips of the cilia is an additional factor essential for pickup (Talbot et al. 1999; Lam et al. 2000). If adhesion is too strong or too weak, oocyte pickup can fail (Lam et al. 2000). Exposure to both mainstream and sidestream smoke increases adhesion (Gieseke and Talbot 2003), which could account for decreased pickup rates even when cilia beat at normal or accelerated rates. Exposure to tobacco smoke adversely affects other adhesive processes involving cells, asbestos, and bacteria (Cantral et al. 1995; Churg et al. 1998; El Ahmer et al. 1999).

Therefore, in vitro studies demonstrate that exposure to tobacco smoke adversely affects oviductal structure and functioning and that nicotine can impair oviductal physiology. Together with in vitro data, in vivo studies demonstrate that maternal smoking adversely affects the oviduct in ways that could impair fertility and complicate pregnancy.

Placenta

Normal Development

Formation of the placenta in the uterus (placentation) is a complex process that is not fully understood. Fetal stem cells (cytotrophoblasts) form a polarized epithelium attached to a basement membrane that surrounds a stromal core containing the placental vasculature and forming chorionic villi. These villi are surrounded by a multinucleate syncytial covering. Floating villi are attached only to the fetal side of the placenta. In contrast, anchoring villi are formed when cytotrophoblastic cells detach from the basement membrane, penetrate the uterine wall, and invade maternal arteries and veins. Cytotrophoblasts travel deeply into uterine arterioles, replace the maternal endothelial lining, and disrupt the smooth muscle wall. This physiological transformation converts the maternal vasculature from a high-resistance,

low-capacitance system to a low-resistance, high-capacitance system that allows for increased blood flow to the fetus. Blood from the spiral arteries then enters the intervillous space where an exchange of substances between the mother and the fetus occurs (Khong 2004). As the placenta develops, the villous system also undergoes remodeling. The terminal villi elongate, and there is a large increase in the peripheral villi capillary volume (Mayhew 2002; Torry et al. 2004). In addition, the thickness of the collective layers of the villi that separate maternal and fetal circulatory systems (villous membrane) decreases, enhancing the exchange of nutrients and metabolic products between the mother and fetus (Mayhew 1998). Interference with the development and remodeling of the placental vasculature likely contributes to adverse pregnancy outcomes.

Effects of Smoking

Studies have examined the effects of maternal smoking on the placenta, but the results are often conflicting. Real or apparent inconsistencies among studies may reflect differences in laboratory techniques and terminology. However, many studies appear to fall into one of three general areas of research: (1) cytotrophoblastic invasion of the uterus and subsequent transformation of uterine blood vessels; (2) development of the fetal capillary and villous system, particularly with respect to whether the placenta can compensate for maternal hypoxia by increasing the supply of oxygen (O_2) and nutrients to the fetus; and (3) transportation of nutrients across the placenta (see "Amino Acids" later in this chapter).

As previously stated, cytotrophoblastic cells in normal pregnancy invade the uterine arterioles and transform them into a high-capacitance system that allows for an increase in blood flow to the fetus. Incomplete transformation of the spiral arteries results in a high vascular resistance in the placenta and a decrease in blood flow to the intervillous space. Studies have described a diminished physiological transformation in placentas from women with preeclampsia (Brosens et al. 1972; Naicker et al. 2003), SAB (Khong et al. 1987), IUGR (Khong et al. 1986), PPROM (Kim et al. 2002), preterm labor (Kim et al. 2003), preterm birth (Kim et al. 2003), or placental abruption (Dommisse and Tiltman 1992).

Physiological transformation appears to be disturbed in women who smoke cigarettes during pregnancy. In vitro studies have shown that formation of cytotrophoblastic cell columns, which is necessary for the invasion of the uterine wall, is disrupted, perhaps from the effects of exposure to nicotine (Genbacev et al. 1995). Among women who smoke, there also appears to be a reduction in the number of cytotrophoblastic stem cells in the floating villi and a reduction in the number of anchoring villi that successfully invade the uterine wall, which may reflect a premature depletion of cytotrophoblastic stem cells (Genbacev et al. 1995). The interference by smoking in cytotrophoblastic invasion of the uterine wall could lead to increased risk of adverse pregnancy outcomes.

Paradoxically, maternal smoking is protective against preeclampsia, which is also characterized by an incomplete transformation of the spiral arteries. As previously noted, an imbalance between proangiogenic and antiangiogenic placental factors may contribute to manifestations of preeclampsia, and smoking may exert its protective effects by affecting this imbalance (Genbacev et al. 2003; Maynard et al. 2003) (see "Pregnancy Complications" earlier in this chapter). In a normal pregnancy, the placenta releases proangiogenic factors, VEGF, and the placental growth factor (PlGF). Placental soluble FMS-like sFlt-1, which is elevated in preeclampsia, is an antagonist of both VEGF and PlGF. An elevated sFlt-1 level is associated with lower levels of VEGF and PlGF and leads to endothelial dysfunction. Maternal smoking appears to increase the placental expression of VEGF-A (Zhou et al. 2002), a major regulator of cytotrophoblastic differentiation along the invasive pathway that is decreased in the preeclamptic placenta. In contrast, studies have found decreased levels of sFlt-1 in the plasma of smokers (Belgore et al. 2000), but not in pregnant smokers (Kämäräinen et al. 2009). The 2004 Surgeon General's report on the health consequences of smoking noted that "the decreased risk of preeclampsia among smokers compared with nonsmokers does not outweigh the adverse outcomes that can result from prenatal smoking" (USDHHS 2004, p. 576). Additional research is needed to confirm or refute the notion that the effects on VEGF-A and/or sFlt-1 explain the reduced risk of preeclampsia in smokers.

Findings on the effects of maternal smoking on the development of the villous capillary system are inconsistent. The fetus of a smoker develops under conditions of reduced partial pressure of O_2, because hemoglobin has an affinity for CO from cigarette smoke that greatly exceeds its affinity for O_2. Thus, the expectation is to see compensatory responses in the placenta similar to those observed with other hypoxic conditions. Such compensatory responses to hypoxia could include increased volume density, branching, and dilation of the fetal capillary system; increased density and proliferation of the cytotrophoblasts; thinning of the villous membrane (Kingdom and Kaufmann 1997; Mayhew 1998; Bush et al. 2000); and increased maternal and fetal hematocrits. Studies have documented increases in both the maternal and fetal hematocrits among smokers (Bodnar et al. 2004), which should lead to increased delivery of O_2 to the fetus.

In addition, several studies have found that the placentas of smokers are heavier or larger than those in

nonsmokers (Naeye 1978; van der Veen and Fox 1982; Howe et al. 1995; Williams et al. 1997), which suggests an expansion of the peripheral villous tree. However, other researchers have found no increase or only a small increase in placental size and/or weight in smokers (Spira et al. 1975; Picone et al. 1982; Demir et al. 1994; Sanyal et al. 1994; Williams et al. 1997; Zhang et al. 1999; Larsen et al. 2002). In many of these studies, the ratio of placental to fetal weight was higher in smokers than in nonsmokers even when the placental weight did not increase. This increase in the ratio of placental to fetal weight could result from a compensatory response to hypoxia, but this explanation has not been established. Studies of morphology have described increase (Pfarrer et al. 1999), decrease (Asmussen 1980; Burton et al. 1989; Teasdale and Ghislaine 1989; Bush et al. 2000; Larsen et al. 2002), and no appreciable difference (van der Velde et al. 1983; Mayhew 1996) in dimensions of the villous capillary system of smokers.

In contrast, studies have consistently shown that maternal smoking is associated with a thickening of the villous membrane, which would decrease the ability of nutrients to diffuse through the placenta (Burton et al. 1989; Jauniaux and Burton 1992; Demir et al. 1994; Bush et al. 2000; Larsen et al. 2002). This increased thickness was attributed to the increased thickness of the trophoblastic component (Jauniaux and Burton 1992; Bush et al. 2000) and to a thickening of the basement membrane (Asmussen 1980; Demir et al. 1994). The thickening of the villous membrane is opposite from an expected compensatory thinning in response to a hypoxic environment and could contribute to fetal growth restriction. Researchers have hypothesized that direct toxic effects of maternal smoking on the placenta are responsible for the thickening of the villous membrane, perhaps due to the accumulation of cadmium that is associated with a reduction in fetal capillary volume (Burton et al. 1989; Bush et al. 2000).

Studies have also explored other effects of maternal smoking on the cellular and noncellular composition of the villous system. Researchers have described changes in placental morphology of smokers, including cytotrophoblastic hyperplasia, focal syncytial necrosis, the loss or distortion of syncytial microvilli, decreased vasculosyncytial membranes, decreased syncytial pinocytotic vesicles, the degeneration of cytoplasmic organelles, increased syncytial knots and decreased syncytial buds, and increased collagen levels in the villous stroma (van der Veen and Fox 1982; van der Velde et al. 1983, 1985; Demir et al. 1994). Evidence of increases in syncytial knots and necrotic areas suggests an increase in syncytial damage among smokers (Demir et al. 1994). However, these effects are not consistent in all studies (Teasdale and Ghislaine 1989; Ashfaq et al. 2003). Researchers have found it difficult to clearly connect these findings with adverse pregnancy outcomes, because the observed changes are not pathognomonic for any particular disorder. However, these cellular and molecular abnormalities of the villous system could lead to an impaired exchange of metabolic products, O_2, and nutrients between the mother and fetus.

Maternal and Fetal Cardiovascular Systems

Smoking acutely increases the heart rate and blood pressure of smokers, particularly after a period of abstinence from smoking (e.g., first cigarette of the day). This finding has led to the suggestion that changes in blood flow may be a mechanism for the lower birth weight observed in infants of smokers. Numerous studies have investigated the effect of cigarette smoke on the cardiovascular system of pregnant women or their fetuses. The studies can be broadly divided into those that examined differences in basal cardiovascular parameters between nonsmokers and smokers after an interval of abstinence and those that examined the acute cardiovascular effects immediately after the pregnant women had each smoked one or two cigarettes. A body of work investigating the relationships of smoking with maternal blood pressure and preeclampsia is not presented here, as preeclampsia is described above.

Seven studies investigated the basal cardiovascular state of mothers who smoked and their fetuses compared with those in a control group of mothers who did not smoke and their fetuses (Table 8.8). Four studies used a radioisotope to study blood flow through the placenta (Table 8.9). In 28 studies, the acute maternal and fetal cardiovascular effects of maternal smoking were examined, and some of these studies also reported baseline differences (Table 8.10). The participants in these 39 studies had healthy singleton pregnancies unless otherwise noted. Most of the percentage differences in the parameters were calculated for this Surgeon General's report from data and graphs in the original articles, but the statistically significant data are those of the original investigators.

Basal Function

Maternal Heart Rate and Blood Pressure During Smoking Abstinence

One prospective study of 203 smokers and 292 nonsmokers at 18, 24, 28, and 34 weeks of gestation found no differences in maternal heart rate during abstinence from smoking (Table 8.8) (Newnham et al. 1990). Two smaller

Table 8.8 Basal maternal and fetal cardiovascular effects of smoking

Study	Design/population	Outcome and comments
MacGillivray et al. 1969	226 women 144 nonsmokers, 82 smokers Gestational age ≥20 weeks Healthy and high-risk pregnancies Lifetime nonsmokers vs. smokers Unadjusted data	**Maternal heart rate:** no data collected; **Maternal blood pressure:** diastolic blood pressure at first prenatal clinic visit during abstinence 9% lower ($p < 0.05$) in smokers than in nonsmokers; systolic blood pressure higher in smokers than in nonsmokers but no significant data or actual numbers given
Phelan 1980	478 women 350 nonsmokers, 128 smokers Gestational age 32–40 weeks All high-risk pregnancies Repeated NST between 32 and 40 weeks' gestation	**NST:** smokers had higher rate of nonreactive NST (21% vs. 13%, $p < 0.005$), but nonreactive NST in nonsmokers stayed nonreactive on subsequent NST; usually, nonreactive NST in a smoker may or may not be nonreactive on subsequent NST
Eldridge et al. 1986	24 women 19 nonsmokers, 5 smokers Gestational age 20–38 weeks	**Fetal heart rate:** no difference in second or early third trimester; heart rate 9% higher in smokers than in nonsmokers ($p < 0.01$) in late pregnancy; **Fetal blood flow: aorta**—significant increase in blood flow (30–50% depending on gestational age) in smokers vs. nonsmokers (no p value given)
Newnham et al. 1990	495 women 292 nonsmokers, 203 smokers Gestational age 18–34 weeks Study conducted at 18, 24, 28, and 34 weeks' gestation	**Maternal heart rate:** no difference between smokers and nonsmokers during abstinence; **Maternal blood pressure:** no difference between smokers and nonsmokers; **Fetal heart rate:** no difference between smokers and nonsmokers; **Blood flow:** no difference in umbilical artery or uteroplacental S/D ratio between smokers and nonsmokers
Matkin et al. 1999	5,369 women 2,478 smokers, 2,891 nonsmokers Gestational age 20–40 weeks	**Maternal blood pressure:** in general, lower mean diastolic blood pressure and higher mean systolic blood pressure in smokers; no significant differences between nonsmokers and any of smokers' groups ($p > 0.05$); no dose-response relationship demonstrable between smoking levels and blood pressure
Lees et al. 2001	5,121 women 4,821 nonsmokers, 800 smokers Gestational age 23 weeks	**Uterine artery:** at pulsatile index of ≥1.45 (≥95% for cohort), likelihood ratio for severe adverse outcome was 5 for nonsmokers and 10 for smokers; odds ratio for severe adverse outcome for smokers, after adjustment for pulsatile index, was 2.18 (95% confidence interval, 1.27–3.74; $p < 0.005$)
Albuquerque et al. 2004	143 women 69 nonsmokers, 74 smokers 28 smokers ≤10 cigarettes/day 45 smokers >10 cigarettes/day Median gestational age 34–35 weeks Abstinence 20 minutes–4 hours before study	**Blood flow: uterine arteries**—no difference in S/D ratio or RI values in smokers vs. nonsmokers; **umbilical arteries**—median S/D ratio 13% greater and median RI value 5% greater in smokers than in nonsmokers ($p < 0.05$); **Fetal middle cerebral artery:** no difference in S/D ratio or RI values between smokers and nonsmokers

Note: Sonographic technology (ultrasound/Doppler) was used to determine maternal heart rate and blood pressure and for fetal monitoring. Prospective studies were conducted during variable periods of abstinence (no smoking before or during protocol). Most percentages were calculated for this Surgeon General's report from data in the study articles or estimated from graphs in the articles. **NST** = nonstress test; **RI** = resistance index; **S/D** = systolic/diastolic.

Table 8.9 Maternal and fetal cardiovascular effects: radioisotope studies of placental intervillous blood flow (IBF) conducted before and after smoking

Study	Design/population	Outcome and comments
Lehtovirta and Forss 1978[a]	12 nonsmokers Gestational age 35–40 weeks	**Xenon^{133} IBF:** overall mean decrease in IBF of 21% ($p < 0.05$) resolved within 15 minutes, but 7 with decreased IBF and 5 with increased IBF
Lehtovirta and Forss 1980[a]	12 healthy women Unknown smoking status 11 women with hypertension (9 nonsmokers, 2 smokers) Gestational age 34–40 weeks	**Maternal heart rate:** mean increase of 15% in both groups ($p > 0.001$); **Maternal blood pressure:** increase of 6% ($p < 0.001$) in healthy women and 2.5% ($p < 0.05$) in women with hypertension; **Xenon^{133} IBF:** baseline IBF 19% lower in women with hypertension; smoking resulted in mean 21% ($p < 0.05$) decrease in IBF in healthy women, and a 28% ($p < 0.05$) increase in IBF in women with hypertension; no change in myometrial blood flow in either group
Rauramo et al. 1983[a]	7 women 1 smoker, 6 nonsmokers Gestational age 37–38 weeks	**Xenon^{133} IBF:** decrease in mean IBF of 20% in 7 measurements ($p < 0.001$), with a concomitant 54% fall in mean differential indices and 45% fall in interval indices; in 5 measurements, an increase in mean IBF, differential indices, and interval indices (24%, p not significant; 37%, $p < 0.001$; and 45%, $p < 0.01$, respectively); relationship between change in IBF and smoking was not clearly stated, but it appears that rise in IBF occurred after fall in IBF; IBF returned to baseline within 20 minutes
Philipp et al. 1984	40 women 20 smokers, 20 nonsmokers Gestational age 32–38 weeks Smokers studied after smoking 2 cigarettes and compared with nonsmokers	**Uteroplacental blood flow measured by indium^{113} labeled transferrin:** significant difference in distribution of scan types ($p < 0.005$); normal flow in 15% of smokers vs. 55% of nonsmokers; intermediate flow in 65% of smokers vs. 30% of nonsmokers; and abnormal flow in 20% of smokers vs. 15% of nonsmokers

[a]Same group of investigators.

studies also found no differences in the maternal heart rate in smokers and nonsmokers at baseline (Table 8.10) (Bruner and Forouzan 1991; Kimya et al. 1998). Many other studies that investigated maternal blood pressure or fetal heart rate did not report data on maternal heart rate, possibly indicating that they did not find a difference between smokers and nonsmokers.

The first study that prospectively evaluated maternal blood pressure during abstinence from smoking found a significantly lower diastolic blood pressure among smokers than among nonsmokers (MacGillivray et al. 1969). Three later studies have not replicated these results (Newnham et al. 1990; Kimya et al. 1998; Matkin et al. 1999). The largest study, of more than 5,000 participants, also found that smokers tended to have a lower mean diastolic blood pressure of 1 to 3 millimeters of mercury, but this difference was not statistically significant and is unlikely to be clinically significant (Table 8.8) (Matkin et al. 1999). The study by Newnham and colleagues (1990) was larger than that by MacGillivray and colleagues (1969) and of a similar design, but no significant differences in maternal diastolic or systolic blood pressure were observed. Two investigations found that the systolic blood pressure of smokers was higher than that of nonsmokers (MacGillivray et al. 1969; Matkin et al. 1999). However, MacGillivray and colleagues (1969) did not report significance data, and Matkin and colleagues (1999) found the difference to be nonsignificant.

Sufficient clinical data indicate that there is no clinically significant difference in the mean maternal heart rate or blood pressure in healthy pregnant nonsmokers and smokers during abstinence from smoking. Further study of the blood pressure distribution, especially in the tails of the statistical distribution, may be warranted, because the percentage of women with blood pressure at or near the hypertensive range is most important clinically, and this group is at a higher risk of adverse pregnancy outcomes (Lees et al. 2001).

Fetal Heart Rate

Four studies provide data on fetal heart rate collected from smokers during periods of abstinence and compared with data from nonsmokers (Eldridge et al. 1986;

Table 8.10 Acute maternal and fetal cardiovascular effects of smoking

Study	Design/population	Outcome and comments
Gennser et al. 1975[a]	12 smokers Gestational age 33–39 weeks	**Maternal heart rate:** median increase of 29% ($p < 0.01$) and correlated with nicotine plasma level; **Fetal heart rate:** no consistent change
Nylund et al. 1979	12 smokers Gestational age 39–45 weeks Hospitalization, high-risk pregnancies Data graphically displayed	**Maternal heart rate:** mean increase of 27% ($p < 0.001$); **Maternal blood pressure:** mean increase in systolic blood pressure of 12% ($p < 0.05$) and in diastolic blood pressure of 14% ($p < 0.05$); **Fetal heart rate:** mean increase of 15% ($p < 0.001$)
Quigley et al. 1979	8 smokers 20–40 cigarettes/day Gestational age 34 weeks	**Maternal heart rate:** mean increase of 35% during smoking and 25% after smoking ($p < 0.01$); **Maternal blood pressure:** mean increase in systolic blood pressure and diastolic blood pressure of 7% and 13%, respectively, during smoking ($p < 0.01$); return to baseline by 20 minutes after smoking; **Fetal heart rate:** mean increase of 17% ($p < 0.01$); return to baseline by 30 minutes after smoking
Barrett et al. 1981	26 smokers Gestational age 31–44 weeks All high-risk pregnancies 1 hour abstinence before testing	**NST:** none developed a nonreactive NST after smoking; **Fetal heart rate:** slight transient increase in 7 smokers, no change in 18 smokers, and decrease in 1 smoker
Kariniemi et al. 1982[a]	N = 8: 1 smoker, 7 nonsmokers Gestational age 37–41 weeks	**Maternal heart rate:** mean increase of 17% ($p < 0.001$); **Maternal blood pressure:** mean systolic blood pressure increase of 11% ($p < 0.001$) and mean diastolic blood pressure increase of 20% ($p < 0.001$); fetal heart rate and variability data not given in relationship to smoking
Forss et al. 1983[a]	8 women 3 smokers, 5 nonsmokers Gestational age 22–26 weeks Data provided only graphically	**Maternal heart rate:** increased ($p < 0.001$); **Maternal blood pressure:** increased ($p < 0.01$); **Fetal heart rate and variability:** heart rate increased ($p < 0.01$); no change in short-term variability, decreased long-term variability
Jouppila et al. 1983	19 smokers Gestational age 29–39 weeks	**Maternal heart rate:** 13% increase ($p > 0.05$); **Fetal heart rate:** 4% increase ($p > 0.05$); **Fetal blood flow: aorta**—no change in diameter, 1% decrease in blood flow (p not significant); **umbilical vein**—no change in diameter, 10% increase in blood flow (p not significant)
Lehtovirta et al. 1983[a]	8 women 1 smoker, 7 nonsmokers Gestational age 37–40 weeks Data provided only graphically	**Maternal heart rate:** increased ($p < 0.001$); **Maternal blood pressure:** increased ($p < 0.001$); **Fetal heart rate and variability:** no significant increase in heart rate but significant decreases in short- and long-term variability
Goodman et al. 1984	10 smokers 10–20 cigarettes/day Gestational age 37–40 weeks Studied on 2 days, smoking on 1 day and no smoking on 1 day Order of smoking randomized	**Fetal heart rate:** increase of 2% with smoking (p not significant); **Fetal heart rate variability:** decrease of 63% in number of accelerations lasting ≥40 minutes ($p < 0.01$)
Kariniemi et al. 1984[a]	8 women Smoking status not given Gestational age 27–32 weeks	**Maternal heart rate:** 24% increase ($p < 0.001$); **Maternal blood pressure:** 9% increase in systolic blood pressure ($p < 0.01$), 15% increase in diastolic blood pressure ($p < 0.001$); **Fetal heart rate and variability:** 11% increase in heart rate ($p < 0.001$), decrease in both DI and II of 24% ($p < 0.001$)

Table 8.10 Continued

Study	Design/population	Outcome and comments
Pijpers et al. 1984	14 smokers Gestational age 34–38 weeks All abstained for 24 hours 7 smoked cigarette and were studied before, during, and after smoking, and 7 abstained Both studied at similar time points	**Maternal heart rate:** 11% increase for smokers (p <0.05) compared with women who abstained; **Maternal blood pressure:** 5% and 10% increase in systolic and diastolic blood pressure, respectively (p <0.05), with no change in women who abstained; **Fetal heart rate:** 4.5% increase (p <0.05) compared with women who abstained; **Fetal blood flow: aorta**—no significant change in velocity or in systolic or diastolic diameters
Sindberg Eriksen et al. 1984	10 smokers Gestational age 34–40 weeks	**Fetal heart rate:** 14% increase (p <0.01), returned to baseline by 20 minutes after smoking; **Fetal heart rate variability:** significant decrease in short-term variability of fetal heart rate but no significant change in long-term variability
Sindberg Eriksen and Gennser 1984	17 smokers Gestational age 33–37 weeks	**Maternal heart rate:** median increase of 42% (p <0.01); **Fetal heart rate:** median increase of 14% (p <0.01); **Fetal blood flow: aorta**—11.6% increase in median diastolic diameter (p <0.01); 13.8% increase in median pulsatile index (p <0.01); significant changes in aorta waveform with smoking; no signs of fetal hypoxia
Sindberg Eriksen and Marsál 1984	30 smokers Gestational age 31–40 weeks	**Maternal heart rate:** median increase of 29% (p <0.01); **Maternal blood pressure:** median increase in systolic and diastolic blood pressure of 14% and 12.5%, respectively (p <0.01); **Fetal heart rate:** median increase of 13% (p <0.01); **Fetal blood flow: aorta**—5% increase in median diameter, 15% increased peak, 20% increased mean blood velocity, and 30% increased flow (p <0.01); **umbilical vein**—9% increase in median diameter, 38.5% increase in flow (both p <0.05), and no change in velocity
Sindberg Eriksen and Marsál 1987[a]	10 smokers Gestational age 33–40 weeks	**Fetal heart rate:** 15% increase (p <0.05), return to baseline by 30 minutes after smoking; **Fetal blood flow: aorta**—5 minutes after smoking, pulsatile index decreased by 9%, and least diastolic blood velocity increased by 22% (p <0.05 for both), but mean peak velocity was unchanged
Sorensen and Borlum 1987	21 smokers 6–20 cigarettes/day Gestational age 24–39 weeks Abstinence 2–12 hours	**Maternal heart rate:** 15% median increase (p <0.05); **Maternal blood pressure:** 8.7% median increase in systolic blood pressure (p <0.05), and no increase in diastolic blood pressure; **Fetal heart rate:** 4.3% median increase (p <0.05); **Blood flow:** no change in fetal cardiac output
Lindblad et al. 1988[a]	24 smokers Gestational age 33–36 weeks	**Maternal heart rate:** 16% increase (p <0.01); **Maternal blood pressure:** no significant increase in systolic blood pressure; 11% increase in diastolic blood pressure (p <0.01); **Fetal heart rate:** 14% increase (p <0.001); **Fetal blood flow: aorta**—11% increase in flow (p <0.01); **umbilical vein**—20% increase in flow (p <0.01)
Morrow et al. 1988	15 smokers Gestational age 36–41 weeks	**Maternal heart rate:** 30% increase (p <0.0001); **Maternal blood pressure:** slight increase in systolic blood pressure (p not significant), and 14% increase in diastolic blood pressure (p = 0.0002); **Fetal heart rate:** 7% increase (p = 0.001); **Blood flow: uterine artery**—increase in S/D ratio (p = 0.08, powered [80%] to detect 17% change in S/D ratio); **umbilical artery**—26% increase in S/D ratio (p = 0.0001)

Table 8.10 Continued

Study	Design/population	Outcome and comments
Bruner and Forouzan 1991	47 women 24 nonsmokers, 23 smokers Gestational age 26–34.5 weeks Smokers studied before and after smoking and compared with control nonsmokers	**Maternal heart rate and fetal heart rate:** no significant difference at baseline between nonsmokers and smokers; no significant change after smoking; **Uterine artery and umbilical artery blood flow:** no significant difference at baseline between nonsmokers and smokers; no difference after smoking in flow velocity waveform or S/D ratio
Graca et al. 1991	51 women Gestational age 34–38 weeks	**Fetal heart rate:** increase of >10 beats per minute in most fetuses after maternal smoking (estimated to be increase of approximately 6–8%) and decreased short-term variability
Castro et al. 1993	19 smokers Mean gestational age 28 weeks ±1 week	**Maternal heart rate:** 27% increase ($p < 0.001$); **Maternal blood pressure:** 8% increase in systolic and 19% increase in diastolic blood pressure ($p < 0.001$); **Uterine artery blood flow:** baseline S/D ratio >90% in 13 subjects; significant decrease in S/D ratio (15%) and resistance index (12%)
Oncken et al. 1996	9 smokers Gestational age 24–36 weeks Percentages estimated from graphs No p value or statement of significance given	**Maternal heart rate:** 17% increase; **Maternal blood pressure:** 4% increase in mean arterial pressure; **Fetal heart rate:** 2% increase; **Blood flow: umbilical artery**—8% decrease in resistance index; **uterine artery**—1% decrease in resistance index
Huisman et al. 1997	5 smokers Gestational age 8–16 weeks (studied weekly) All twin pregnancies	**Maternal heart rate:** 12% increase ($p < 0.005$); **Maternal blood pressure:** no significant change; **Fetal heart rate:** no significant change; **Umbilical artery blood flow:** 13% increase in pulsatile index ($p < 0.01$)
Oncken et al. 1997	15 smokers Gestational age 24–34 weeks Overnight abstinence, then studied every 2 hours during 8 hours of ad lib smoking	**Maternal heart rate:** significant increase 2 hours after baseline ($p < 0.001$); **Maternal blood pressure:** approximately 6% mean increase in systolic blood pressure at 2 hours after baseline ($p < 0.001$); **Fetal heart rate:** 0.7% increase (p not significant); **Fetal heart rate reactivity:** nonsignificant change: 1 loss of reactivity at 4 hours, 2 gained reactivity, and 12 had no change in reactivity; **Blood flow: fetal MCA**—2.4% decrease in resistance index at 4 hours ($p = 0.02$); **umbilical artery**—3% increase in resistance index at 2 hours (significance not stated); **uterine artery**—nonsignificant fluctuation (decrease and increase) in mean resistance index over 8 hours
Kimya et al. 1998	43 women 22 smokers, 21 nonsmokers Gestational age 20–40 weeks Smokers studied before and after smoking and compared with control nonsmokers	**Maternal heart rate:** no difference at baseline between smokers and nonsmokers, 23% increase ($p < 0.01$) after smoking; **Maternal blood pressure:** no difference at baseline, systolic and diastolic blood pressure increased by 11% and 23%, respectively, after smoking ($p < 0.05$); **Blood flow: uterine artery**—at baseline, smokers had 12% lower S/D ratio, 19% lower pulsatile index, and 19% lower resistance index compared with those of smokers ($p < 0.001$); after smoking—no significant change in S/D ratio, pulsatile index, or resistance index; **umbilical artery**—no difference in pulsatile index or resistance index for nonsmokers vs. smokers at baseline and after smoking; S/D ratio was 13% lower in nonsmokers, with no change in S/D ratio after smoking

Table 8.10 Continued

Study	Design/population	Outcome and comments
Coppens et al. 2001	26 women 13 smokers, 13 nonsmokers >10 cigarettes/day Mean gestational age 37–38 weeks Smokers studied before and after smoking and compared with control nonsmokers	**Fetal heart rate:** no difference between smokers and nonsmokers at baseline or after smoking; **Fetal heart rate variability:** at baseline, percentage of time in high variability was 31% lower in smokers than in nonsmokers ($p < 0.05$); similar differences remained after smoking; **Blood flow: umbilical artery**—at baseline, no difference in pulsatile index between nonsmokers and smokers; pulsatile index increased by 76% after smoking ($p < 0.05$)
Oncken et al. 2002	15 smokers Gestational age 29–36 weeks Smoked 1 cigarette/hour for 8 hours Studied at baseline and at 4 hours, just before 4th cigarette	**Fetal heart rate:** baseline vs. 4 hours—no change ($p = 0.17$); **Fetal heart rate variability**: **nonstress test**—20% were nonreactive at baseline, and 73% were nonreactive at 4 hours ($p = 0.013$); nonreactive tests at baseline were also nonreactive at 4 hours; all fetuses demonstrated fetal breathing movements indicating fetal well-being
Ates et al. 2004	67 smokers Gestational age 32–40 weeks	**Maternal heart rate:** 6% increase ($p < 0.001$); **Maternal blood pressure:** no significant increase ($p = 0.2$); **Fetal heart rate:** no significant increase; **Fetal heart rate variability:** 7% decrease in variability ($p = 0.08$) and 19% decrease in number of accelerations ($p = 0.035$); **Blood flow: umbilical artery and fetal MCA**—no significant change in resistance index, pulsatile index, S/D ratio, or maximum and minimum velocity

Note: Maternal heart rate and blood pressure measurements and fetal monitoring used sonographic technology (ultrasound/Doppler). Prospective studies were conducted before and after smoking. Unless noted otherwise in description of study design, the following applies to all studies: overnight (>8 hours) smoking abstinence before smoking study cigarette. All were healthy singleton pregnancies, and percentage changes are for means. Most percentages were calculated for this Surgeon General's report from numeric data in article or estimated from graphs. **DI** = differential index or indices; **II** = interval index or indices; **MCA** = middle cerebral artery; **S/D** = systolic/diastolic.
[a]Same group of investigators.

Newnham et al. 1990; Bruner and Forouzan 1991; Coppens et al. 2001). These studies found no differences except for the small study by Eldridge and colleagues (1986). This study compared 19 nonsmokers with 5 smokers and found no difference in fetal heart rate during the second or early third trimester, but it did find a significant 9-percent elevation among smokers late in the pregnancy.

Acute Effects of Smoking

Maternal Heart Rate

Twenty-one studies provide data on the immediate effect of smoking one or two cigarettes on maternal heart rate (Table 8.10). The general design of these studies is similar. Healthy active smokers with singleton births in the latter half of pregnancy abstained from smoking overnight and were then studied before and after smoking one or two cigarettes. The different designs of the studies are noted in Table 8.10. All but two of the studies (Jouppila et al. 1983; Bruner and Forouzan 1991) found a statistically significant transient increase in maternal heart rate immediately after smoking. The largest study, with 67 pregnant smokers, found a significant increase of 6 percent in maternal heart rate (Ates et al. 2004), and one smaller study, with 17 pregnant smokers, found a significant 42-percent increase in maternal heart rate immediately after smoking (Sindberg Eriksen and Gennser 1984). Other increases in maternal heart rate ranged from 10 to 30 percent, which were similar to the nonsignificant effect in the study conducted by Jouppila and colleagues (1983).

Sufficient clinical data establish that smoking a cigarette after a period of abstinence transiently elevates maternal heart rate, although the magnitude of the increase varies. This finding holds true even when studies involving nonsmokers are excluded. Only one study (Oncken et al. 1997), however, addressed the effect of ad lib smoking throughout the day on maternal heart rate. Oncken and colleagues (1997) found a maximal increase of 11 beats per minute in maternal heart rate two hours after

baseline—an increase of approximately 13 percent—with ad lib smoking. The clinical significance of a transiently elevated maternal heart rate during pregnancy is unknown.

Maternal Blood Pressure

Of the 16 studies that examined the acute effects of smoking on maternal blood pressure (Table 8.10), all but 2 (Huisman et al. 1997; Ates et al. 2004) reported a transient but significant elevation in the mean or median diastolic or systolic blood pressure or in the mean arterial pressure. The largest increases, ranging from 10 to 23 percent, were observed for diastolic blood pressure, but most studies found an increase of less than 15 percent. The largest study, with 67 participants, found a small but nonsignificant increase in diastolic blood pressure after smoking (Ates et al. 2004). In general, the acute effect of smoking on maternal systolic blood pressure was less than the effect on diastolic blood pressure. Three studies reported no significant increase in systolic blood pressure, and the largest study found a small but nonsignificant ($p = 0.2$) increase (Table 8.10) (Ates et al. 2004). In the remaining 10 studies, transient increases ranged from 5 to 14 percent. These data indicate that smoking after abstinence transiently increases diastolic blood pressure and, to a lesser extent, systolic blood pressure. Because one large study (Ates et al. 2004) found a nonsignificant effect of smoking on maternal blood pressure, additional large studies may be needed.

The release of catecholamine may mediate the elevations in maternal heart rate and blood pressure reported in these studies. In a study of pregnant women, smoking was associated with an acute rise in plasma levels of norepinephrine, epinephrine, and dopamine and an associated acute rise in maternal heart rate and blood pressure (Quigley et al. 1979).

Fetal Heart Rate

Twenty-five studies (Table 8.10) collected data on fetal heart rate before and after mothers smoked one or two cigarettes. Ten studies, including the largest study (Ates et al. 2004) and two studies with a control group of nonsmokers (Bruner and Forouzan 1991; Coppens et al. 2001), found no effect of smoking on fetal heart rate. Five studies reported that smoking after abstinence was associated with a 2- to 8-percent transient increase in fetal heart rate, and eight studies reported an increase of 11 to 17 percent in fetal heart rate. The studies that found mean elevations in the fetal heart rate above 10 percent were conducted between 1979 and 1988. The five studies published after 1996 reported no statistically significant difference.

Variability in Fetal Heart Rate

Healthy fetal heart rate is variable, and there are short- and long-term patterns to this variability. The "differential index" is an alternative term for short-term variability, and the "interval index" is an alternative term for long-term variability. Healthy fetal heart rate also has episodes of accelerations. Researchers use the variability in fetal heart rate and the presence of episodes of accelerations to measure fetal well-being. This variability and acceleration are measured in the noninvasive nonstress test (NST). A reactive NST is a sign of fetal well-being, and a nonreactive NST is a sign of fetal distress. The NST is routinely used in the third trimester of pregnancy and during labor to monitor high-risk pregnancies and to assess low-risk pregnancies if concerns develop. Healthy fetuses have transient periods of decreased variability and accelerations, which would appear as a transiently nonreactive NST.

Data on fetal heart rate reactivity and accelerations and the NST are presented in Tables 8.8 and 8.10. A large cohort of mothers with high-risk pregnancies among smokers and nonsmokers was studied repeatedly over the course of the pregnancies (Table 8.8) (Phelan 1980). Although smokers had a high rate of nonreactive NSTs, many were reactive at a subsequent visit. A nonreactive NST in a pregnant smoker should generally be repeated to rule out a false nonreactive result. There are no reports on the prevalence of nontransient, abnormal NSTs among healthy smokers versus healthy nonsmokers.

Nine studies investigated variability in fetal heart rate before and after maternal smoking (Table 8.10). All but two studies (Oncken et al. 1997; Coppens et al. 2001) found that either maternal smoking transiently decreased short- and long-term variability or the NST became nonreactive. Three of the studies with positive findings were conducted with a control group of nonsmokers by the same team of investigators (Forss et al. 1983; Lehtovirta et al. 1983; Kariniemi et al. 1984). One investigator did not find a loss of reactivity from smoking one cigarette but did find a large increase in nonreactive NSTs with additional smoking (Oncken et al. 2002). The two largest studies (Graca et al. 1991; Ates et al. 2004) found a significant decrease in variability of fetal heart rate and an accelerated increase after smoking.

These data indicate that maternal smoking transiently decreases variability of fetal heart rate. However, the clinical significance of these transient decreases in the heart rate of fetuses of smokers is not clear. Generally, the nontransient changes in these parameters are the clinically important changes.

Blood Flow in Uterus, Placenta, and Fetus

Table 8.9 presents data on blood flow in the uterus and placenta from studies that used a radioisotope, which is considered the "gold standard" because it directly measures flow. The development of fetal sonographic technology has replaced radioisotope studies, because radioisotopes measure only maternal blood flow and expose the mother and fetus to radiation. Sonography is noninvasive and can be used to assess blood flow in both the fetus and the mother. Table 8.10 presents data from ultrasound and Doppler sonography on vessel diameter and blood velocity. These data are then used to calculate blood flow, which is difficult to measure, because the sonographer must visually mark the diameter of a vessel. Flow is proportional to the fourth power of the vessel radius, so even very small changes in the measurement of the diameter have a large effect on the calculation. Furthermore, all of the studies are unblinded. Sonographic technology advanced greatly between 1978 and 2004, which may partly explain the variations in the results from more than 25 years of publications, as is discussed here.

The most commonly used surrogate measures of blood flow are the ratio of systolic to diastolic blood flow velocity (S/D ratio), pulsatile index, and resistance index (RI). The S/D ratio is defined as the ratio of the time-averaged maximal systolic and diastolic blood flow velocities. The pulsatile index is defined as the difference between peak velocity and the lowest diastolic velocity, divided by the mean velocity during the heart cycle. The RI is defined as the difference between the maximal systolic and diastolic flow velocities, divided by the systolic flow velocity.

Independent of studies of smoking, researchers have used the S/D ratio, pulsatile index, and RI measures to monitor high-risk pregnancies and to predict outcomes (Maulik et al. 1990; Alatas et al. 1996; Fong et al. 1999; Özeren et al. 1999; Coleman et al. 2000; Gudmundsson et al. 2003; Axt-Fliedner et al. 2005; Li et al. 2005). In low-risk pregnancies, these measures of blood flow are not sensitive to or specific predictors of adverse outcomes such as preeclampsia or IUGR (Kurmanavichius et al. 1990; Irion et al. 1998; Albaiges et al. 2000; Harrington et al. 2004; Schwarze et al. 2005), except when the measures are markedly abnormal (Becker et al. 2002; Papageorghiou et al. 2005).

Four radioisotope studies of placental intervillous blood flow (IBF) have been performed (Table 8.9). Historically, these four studies have provided the initial data for the hypothesis that decreased maternal blood flow through the placenta caused fetal growth retardation. Three studies by the same group of investigators used xenon^{133} to determine the acute effect of smoking on IBF among nonsmokers (Lehtovirta and Forss 1978, 1980; Rauramo et al. 1983). The results of the three studies are contradictory. Smoking resulted in either an acute increase or a decrease in IBF, depending on the patient and the study. The fourth radioisotope study used indium^{113}-labeled transferrin to compare smokers immediately after smoking with nonsmokers (Philipp et al. 1984). This study found a significant difference in the distribution of normal and abnormal blood flow between the two groups, and a smaller proportion of scans were normal among the smokers.

Of four studies conducted during abstinence from smoking, two found no difference in blood flow parameters of uterine arteries between nonsmokers and smokers (Table 8.8) (Newnham et al. 1990; Albuquerque et al. 2004); the latter did find a difference in blood flow in umbilical arteries, but the larger Newnham study did not. The very small study by Eldridge and colleagues (1986) found an increase in aortic blood flow. The most important study found an association between the risk of a severe adverse pregnancy outcome and a pulsatile index for the uterine artery that doubled among smokers (Lees et al. 2001).

Sixteen studies used Doppler sonography to examine blood flow parameters in maternal smokers and their fetuses before and after smoking or in smokers after smoking compared with nonsmokers and their fetuses (Table 8.10). Four studies by the same group of investigators found that smoking dramatically increased four parameters (blood flow, velocity, diameter, and pulsatile index) (Sindberg Eriksen et al. 1984; Sindberg Eriksen and Marsal 1984, 1987; Lindblad et al. 1988). One study found an acute 76-percent increase in the pulsatile index of the umbilical artery after maternal smoking (Coppens et al. 2001). Six studies found no effect on blood flow, velocity, diameter, S/D ratio, pulsatile index, and RI in the uteroplacental or fetal blood vessels (Jouppila et al. 1983; Pijpers et al. 1984; Sorensen and Borlum 1987; Bruner and Forouzan 1991; Kimya et al. 1998; Ates et al. 2004). However, Oncken and colleagues (1997) found a negligible change, and four studies found either increases or decreases in the S/D ratio and the pulsatile index (Table 8.10) (Morrow et al. 1988; Castro et al. 1993; Oncken et al. 1996; Huisman et al. 1997).

In summary, differences between blood flow in smokers during abstinence and that in nonsmokers do not appear to be significant. However, the study by Lees and colleagues (2001) raises concerns because it indicates that with an elevated pulsatile index in the uterine artery, maternal cigarette smoking doubles the risk of a severe, adverse pregnancy outcome. The data on the acute effects of smoking on maternal and fetal blood flow are more

contradictory, and no generalizations can be made at this time.

Fetal Tissue and Organogenesis

Timing and Critical Periods

The embryonic period includes the first eight weeks after fertilization and constitutes a significant period in human development. During this time, all major internal and external structures start to develop, involving many complex interactions that must occur in an orderly sequence. The embryonic period is a time of rapid differentiation, and the developing organs are particularly susceptible to the effects of exogenous agents. The stage of embryonic development determines the embryo's susceptibility to unfavorable environmental factors. The embryo is most easily disturbed during the organogenesis period, from day 15 to day 60 after conception. In addition, each system or organ of an embryo has a critical period when its development may be altered. The effects of some environmental toxins on the developing embryo and fetus can be direct and lethal or subtle with delayed but serious consequences. Thus, multiple factors are involved in identifying and evaluating the effects of exposure to tobacco smoke on the developing baby.

Evidence on Effects of Smoking

Some epidemiologic studies report an association between maternal smoking and various congenital malformations. In this area of research, the associations with smoking most frequently examined and published relate to nonsyndromic orofacial clefting, congenital heart disease, malformations of the lower extremities such as clubfoot or limb deficiency defects, hypospadias, gastroschisis, and craniosynostosis (see "Birth Defects" earlier in this chapter). Data supporting a causal association between nonsyndromic orofacial clefting and maternal smoking have strengthened, but few studies have addressed possible pathogenetic mechanisms.

Traditionally, investigators have used animal models and postmortem tissues to detect the effects on organogenesis of exposure to tobacco smoke by conducting gross morphologic, soft tissue, and skeletal examinations. Early studies of this type involving exposure to mainstream cigarette smoke provide little data supporting an effect on organogenesis. Of seven studies, four did not find any effects (Wagner et al. 1972; Reznik and Marquard 1980; Peterson et al. 1981; Bassi et al. 1984), and three mentioned limited findings but lacked sufficient details for a full evaluation (Schoeneck 1941; Tachi and Aoyama 1983; Amankwah et al. 1985).

A subsequent set of experiments exposed pregnant Wistar rats to sidestream cigarette smoke, and the pups were then examined for gross morphologic changes (Table 8.11). Researchers observed a dose-dependent reduction in birth weight ($p < 0.001$) but no increase in macroscopically visible gross anomalies (Nelson et al. 1999a). Ossification was delayed throughout the skeleton in all exposed groups regardless of the dose. The second part of the experiment studied the histopathologic changes in tissues such as the lung, liver, stomach, kidney, and intestines (Nelson et al. 1999b). The lung tissues of pups of dams exposed to smoke showed increased apoptosis, mesenchymal changes, and hyperplasia of bronchial muscles. Researchers found abnormal hematopoiesis, proliferation of bile ducts in the liver, and delayed maturation of the glomeruli, gastric epithelia, and intestinal villi. Another study exposed Sprague-Dawley rats to mainstream tobacco smoke by nose-only inhalation (Carmines et al. 2003). Males were exposed four weeks before and during mating, and females were exposed two weeks before and during mating and through gestational day 20. Exposure to tobacco smoke was confirmed by biomarker evaluation. Researchers evaluated external and internal abnormal macroscopic findings, histopathology of the placenta and fetal tissue, and skeletal radiograms. They concluded that exposure to tobacco smoke was not associated with any congenital malformations in the offspring. However, numerous abnormalities were described, including hypoplasia of the internal genital structures in the exposed adult male rats and decreased ossification in the fetuses of the exposed dams.

Epidemiologic studies show that offspring of maternal smokers have abnormal lung function and associated higher incidences of lower respiratory disorders. The identification of nicotinic acetylcholine receptors in fetal lung suggests a mechanism that may underlie the observed postnatal pulmonary abnormalities. This hypothesis was tested in monkeys to determine whether maternal exposure to nicotine would produce changes in lung function or morphology in newborn monkeys similar to the changes observed in human infants (Sekhon et al. 2001). Pregnant rhesus monkeys were infused with either nicotine comparable to heavy smoking in humans (1.5 milligrams per kilogram per day [mg/kg/day], $n = 7$) or saline ($n = 7$) timed to days 26 through 160 of gestation. The fetuses were delivered by cesarean section and on the next day had pulmonary function testing. They were then sacrificed, and their lungs were weighed and fixed. There was a significant decrease in fetal lung weight (16 percent) and fixed lung volume (14 percent) after in utero exposure to nicotine. All lung function tests (e.g., peak tidal expiratory volume, mean mid-expiratory volume, and forced expiratory volume at peak expiratory flows) were also

Table 8.11 Animal and in vitro studies on association between maternal smoking and congenital abnormalities with relevant genetic and/or molecular hypotheses

Study	Design	Developmental defect studied	Proposed mechanism or hypothesis	Findings
Nelson et al. 1999a	Pregnant Wistar rats were exposed to sidestream cigarette smoke (13 mg of tar, 0.9 mg of nicotine) Pups examined	Histopathologic changes in tissues such as lung, liver, stomach, kidney, and intestines	Define microscopic morphology after exposure to cigarette smoke	• Lung tissues showed increased apoptosis, mesenchymal changes, and hyperplasia of bronchial muscles • Abnormal hematopoiesis and proliferation of bile ducts in liver • Delayed maturation of glomeruli, gastric epithelia, and intestinal villi
Nelson et al. 1999b	Pregnant Wistar rats were exposed to sidestream cigarette smoke (13 mg of tar, 0.9 mg of nicotine) Pups examined	Gross morphologic changes in tissues	Define macroscopic morphology after exposure to cigarette smoke	• Dose-dependent reduction of birth weight was observed ($p < 0.001$) • No macroscopically visible gross anomaly was observed • Delay in ossification in entire skeleton was observed in all exposed, regardless of dose
Subramaniam et al. 1999	12-week-old female Sprague-Dawley rats exposed to sidestream cigarette smoke and unexposed control group 2 weeks after exposure, females inseminated by unexposed males Exposure continued during mating and pregnancy Pups from the exposed group continued to be exposed to cigarette smoke until examination	Lung surfactant proteins SP-A and SP-B in bronchoalveolar lavage fluids, preparations from postnatal days 1, 3, 7, 14, 21, and 35 from sham- and smoke-exposed pups	Determine whether perinatal exposure to sidestream cigarette smoke decreases amount of surfactant in developing rat pups	• Smoke-exposed pups showed reduced level of SP-A on day 1 and a higher level of SP-A and phospholipids on day 21 • Perinatal exposure to sidestream smoke can have deleterious effects on the developing lung

Table 8.11 Continued

Study	Design	Developmental defect studied	Proposed mechanism or hypothesis	Findings
Panter et al. 2000	27 Spanish-type female goats and 28 Western ewes were given *Nicotiana glauca* during pregnancy Concentration of anabasine in the dried plants was 0.175–0.23% vs. 2.4% in extracts Treatment groups divided into 6 subgroups and fed dried plant by gavage or anabasine-rich extract by capsule on days 32 and 41 of gestation Low initial dose (5–8 mg/kg) titrated until clinical signs of toxicity (maximum 18 mg/kg)	Intrauterine growth, presence or absence of CP, facial asymmetry, and skeletal contractures in newborn goats and sheep	Compare incidence of plant-induced CP formation between sheep and goat treatment groups Determine differences in sensitivity of study animals exposed to same toxicants in similar doses and manner Determine other variables that might explain cause of CP formation	• Fetuses from both study groups showed decreased fetal movement, as determined by ultrasound • 21 of 45 (47%) newborn goats exposed during the early part of pregnancy (32–41 gestational days) had CP; only 1 of 45 (2.2%) had skeletal contractures • 1 of 35 (2.9%) lambs had CP, and 6 of 35 (17%) had contractures • Goat model is more efficient and reliable for studying CP deformities • Tobacco plant and its extract is a well-established cause of congenital cleft formation • Exact mechanism is undetermined, but neuromuscular blockade may play a role
Sekhon et al. 2001	Pregnant rhesus monkeys received subcutaneous infusion of nicotine (1.5 mg/kg per day (n = 7) or saline (n = 7) on gestational days 26–160 After delivery by cesarean section, fetuses had pulmonary function testing on next day	Pulmonary function before and after exposure to nicotine Lung weight and morphology	Interaction of nicotine with nicotinic acetylcholine receptor in developing lung responsible for altered pulmonary morphogenesis and mechanics observed in human infants whose mothers smoked during pregnancy	• Lung weight and fixed lung volume were significantly decreased in exposed monkeys (16% and 14%, respectively) • Fixed lung volume and lung volume normalized to body weight decreased (14%, p = 0.001; 11%, p = 0.006, respectively) • All lung function test results were significantly lower in newborns exposed to nicotine during gestation

Table 8.11 Continued

Study	Design	Developmental defect studied	Proposed mechanism or hypothesis	Findings
Carmines et al. 2003	Potential developmental effects of smoke from 1R4F reference cigarette examined Sprague-Dawley rats exposed for 2 hours/day, 7 days/week, by nose-only inhalation at target mainstream smoke levels of 150, 300, and 600 mg/m^3 of total particulate matter Males exposed 4 weeks before and during mating and females for 2 weeks before and during mating and through gestational day 20 Sham controls: filtered air to simulate nose-only exposure, and cage controls maintained untreated Smoke exposure confirmed through biomarker evaluation	Histopathologic evaluation of placenta and fetal tissue External and internal malformations Macroscopic and microscopic findings and changes determined by skeletal radiograms	Parental exposure to tobacco smoke may cause congenital malformations	• Exposure duration was limited • Number of study and control subjects was inadequate • Morphologic and dysmorphic evaluations were not thorough • Adult male rats showed hypoplasia of the internal male genital structures • Fetuses from dams exposed to tobacco smoke showed decreased ossification • Possibility of an association
Lavezzi et al. 2004	54 sudden and unexplained fetal and infant deaths (13 stillbirths, 7 neonatal deaths, and 34 from sudden infant death syndrome) Postmortem examination 33 (61%) nonsmokers 21 (39%) smokers Sections of brainstem and medulla oblongata examined with morphometric methods	Brainstem histopathology and morphometric analysis of arcuate nucleus	Decrease in size of the arcuate nucleus of brainstem was expected in group exposed to smoke	• Mothers of 18 of 34 infants with hypoplasia in >20% of arcuate nucleus were smokers ($p < 0.05$) • Homeobox-containing gene • *EN2* is a candidate gene thought to regulate development of arcuate nucleus • Cigarette smoke may directly affect this gene, resulting in brainstem abnormalities

Table 8.11 Continued

Study	Design	Developmental defect studied	Proposed mechanism or hypothesis	Findings
de la Chica et al. 2005	Prospective study 25 control mothers and 25 mothers who smoked (≥10 cigarettes/day for ≥10 years) Amniocytes obtained by routine amniocentesis for prenatal diagnosis Questionnaire about smoking patterns used	Chromosomal instability in routine chromosome spreads Breakpoints implicated in chromosomal abnormalities identified by G-banding	Determine whether maternal smoking has a genotoxic effect on amniotic cells, expressed as increased chromosomal instability Analyze whether any chromosomal regions are especially affected by tobacco	• Smoking as defined and during pregnancy is associated with increased chromosomal instability in amniocytes • Band 11q23, known to be involved in leukemogenesis, seems especially sensitive to genotoxic compounds contained in tobacco
Ejaz et al. 2005	Chicken embryo model In vivo examination of neonatal development Effects examined of different preparations of nicotine and solutions of whole mainstream smoke on embryonic movements during neonatal development Activity level before and after exposure to nicotine was measured	Kinematic analysis	Evaluate effects of nicotine and solutions of whole mainstream smoke on embryonic movements	• Low doses of nicotine induced hyperactivity, and higher doses induced hypoactivity • Significant ($p < 0.01$) decrease in movements with application of 10 mg of nicotine and different preparations of solution of whole mainstream smoke

Table 8.11 Continued

Study	Design	Developmental defect studied	Proposed mechanism or hypothesis	Findings
Lavezzi et al. 2005	Postmortem examination of 42 stillborn babies In 30 of 42, no cause of death was identified (sudden intrauterine unexplained death) In 12 of 42, causes of death were known (intrauterine explained death) 15 sudden intrauterine unexplained deaths and 1 intrauterine explained death of infants born to maternal smokers 16 maternal smokers 25 nonsmokers Brainstems studied by immunohistochemistry to assay expression of *EN2* gene, somatostatin, and tyrosine hydroxylase enzyme	Brainstem histopathology/ morphology	Morphologic changes of brainstem expected in the smoke-exposed group	• Brainstem sections from 13 stillbirths with no definite cause of death but with known exposure to tobacco smoke showed varying degrees of hypoplasia of arcuate nucleus and abnormal staining pattern with antibodies applied • Exposure in utero to maternal smoking may strongly interfere with brain biologic parameters, including decrease in noradrenergic activity in brainstem

Note: **CP** = cleft palate; **mg** = milligrams; **mg/kg** = milligrams per kilogram; **mg/m^3** = milligrams per cubic meter.

significantly lower in the newborns exposed to nicotine, demonstrating that prenatal exposure to nicotine compromises lung growth and pulmonary function. Although there was no histopathologic description of the examined lungs, researchers have described changes in lung morphology in humans (DiFranza et al. 2004), as well as in rats (Nelson et al. 1999b). Another experiment exposed female Sprague-Dawley rats aged 12 weeks to tobacco smoke and then mated them to unexposed males. Postnatal measurements of the pups' lung surfactant levels of protein (SP-A and SP-B) in bronchoalveolar lavage fluids showed a reduced level of SP-A on day 1 and a higher level of SP-A and phospholipid on day 21 among pups exposed to smoke (Subramaniam et al. 1999).

One study examined the induction of cleft palate by *Nicotiana glauca* (wild-tree tobacco) or anabasine-rich extracts during the first trimester of pregnancy and compared Spanish-type goats with crossbred Western-type sheep (Panter et al. 2000). Bilateral cleft palate was induced in 100 percent of the embryonic and fetal goats by gavage of the pregnant mothers with anabasine-rich extracts. Eleven percent of the newborn goats showed extracranial abnormalities, mainly contractures of the metacarpal joints, in addition to bilateral cleft palate. Most of these contractures resolved spontaneously within four to six weeks after delivery. In contrast, only two lambs from ewes exposed to both substances had cleft palate. However, all lambs exposed to both substances had contractures, which indicated differential susceptibility of the species. The researchers postulated that an alkaloid-induced reduction in fetal movement during the period of normal palate closure caused the cleft palate and the multiple flexion contractures. This postulation is supported by a later study that used the chick embryo model to conduct an in vivo examination of the effects of different preparations of solutions of nicotine and of mainstream whole smoke on embryonic movements during neonatal development (Ejaz et al. 2005). In this experiment, low doses

of nicotine induced hyperactivity and high doses induced hypoactivity. Accordingly, there was a significant ($p < 0.01$) decrease in movements after applying 10 mg of nicotine and different preparations of whole mainstream smoke solutions. The decrease in embryonic movements was dose dependent and did not resolve by the end of the experiment. The researchers concluded that nicotine could alter embryonic movements that are important during embryogenesis for the differentiation and maturation of the body systems.

In a clinical study, researchers collected amniocytes from routine amniocenteses of 25 control women and 25 women who smoked (≥10 cigarettes per day for ≥10 years). Amniocytes of the smokers showed increased chromosomal instability; breakpoints involving band 11q23, which is commonly implicated in hematopoietic malignancies, was the chromosomal region most affected (de la Chica et al. 2005). Another study examined autopsy specimens from 42 stillborn infants (Lavezzi et al. 2005). Researchers studied the brainstem tissue by immunohistochemistry to evaluate the expression of the *EN2* gene, somatostatin, and the tyrosine hydroxylase enzyme. Brainstem sections from stillborn infants whose mothers had smoked during pregnancy showed hypoplasia of the arcuate nucleus and an abnormal staining pattern with the antibodies applied. Thus, in utero exposure to maternal smoking may strongly interfere with brain biologic parameters of brain development, including a decrease in the noradrenergic activity in the brainstem, resulting in pulmonary hypodevelopment and even an apparently unexplained sudden death of the fetus (Lavezzi et al. 2005).

Researchers have identified an increasing number of polymorphisms of genes encoding drug- and/or toxin-metabolizing enzymes, transporters, and receptors. Some of these genetic factors have a major impact on drug sensitivity, adverse reactions, or variations of responses to environmental toxins. As a result, many investigators have studied polymorphisms of certain candidate genes to elucidate the pathogenesis of the effects of maternal smoking on the developing embryo and fetus (see "Smoking and Maternal and Neonatal Genetic Polymorphisms" later in this chapter).

Investigators have proposed other mechanisms for the adverse effects of smoking on organogenesis, particularly orofacial clefting. CO contributes to fetal hypoxia, which investigators have associated with an increased risk for cleft lip and cleft palate in susceptible strains of mice (Millicovsky and Johnston 1981; Bronsky et al. 1986; Bailey et al. 1995). Impaired uteroplacental circulation may result in a reduced supply of essential nutrients for embryonic tissues (van Rooij et al. 2001). Studies have associated poor intake of vitamin B_6 and multivitamins with a risk of oral clefts (Botto et al. 2004; Munger et al. 2004). Other possible mechanisms include (1) reductions in serum folate levels mediated by maternal smoking (McDonald et al. 2002; Mannino et al. 2003; Ortega et al. 2004), (2) exposure to cadmium that is present in increased amounts in the placentas of smokers (Ronco et al. 2005) and is associated with teratogenic effects in certain rats (Ferm 1971; Chernoff 1973), and (3) DNA damage by PAHs (Lammer et al. 2004; Perera et al. 2004). Further work is needed to elucidate the extent to which these or other mechanisms involving the complex mixture of chemicals in cigarette smoke account for the increased risk of oral clefts.

Immune System

Cigarette smoking is associated with an increased risk for many types of infectious diseases including pneumococcal pneumonia, Legionnaires' disease, meningococcal disease, influenza, the common cold, and infection with *Helicobacter pylori* (Arcavi and Benowitz 2004). In addition, studies have associated smoking with seropositivity for human immunodeficiency virus (HIV) and an increase in the transmission of HIV from infected mothers to their offspring (Boulos et al. 1990; Royce and Winkelstein 1990; Burns et al. 1991, 1994).

The mechanisms through which smoking increases the risk of infection are not well defined and are likely complex, involving both innate and adaptive immune responses. Compared with nonsmokers, smokers appear to have a leukocytosis (Corre et al. 1971; Friedman et al. 1973; Yeung and Buncio 1984; Hughes et al. 1985; Calori et al. 1996; Jensen et al. 1998a) and elevations in levels of all major blood cell types (Corre et al. 1971). This leukocytosis could be a result of nicotine-induced increases in the release of catecholamine (Friedman et al. 1973). However, the consequences of an increased white blood cell count are unclear. It appears that there are increases in both CD4+ (an HIV-helper white blood cell) and CD8+ (an HIV-suppressor white blood cell) T-cell populations in smokers, although heavy smokers may have reduced CD4+ cell counts, and effects may vary by race (Sopori 2002; Arcavi and Benowitz 2004). A decline in CD4+ cell counts could contribute to a decrease in B-cell proliferation and immunoglobulin (Ig) synthesis, which would increase the risk of infection (Arcavi and Benowitz 2004). However, in a study of pregnant smokers compared with pregnant nonsmokers, a decline in CD4+ count was not described (Luppi et al. 2007).

In general, smoking appears to have immunosuppressive effects. For example, lymphocytes in smokers appear to have a decreased response to T-cell mitogens (Sopori 2002), and polymorphonuclear leukocytes show decreases in chemotaxis and migration (Noble and Penny

1975; Corberand et al. 1979), which do not appear to be attributable to exposure to nicotine (Sasagawa et al. 1985). Study findings suggest that smokers have reduced titers of antibodies to the influenza virus and low serum levels of all Ig classes except IgE (Gerrard et al. 1980; Sopori 2002). In addition, smoking may increase levels of autoantibodies, perhaps contributing to some autoimmune disorders (Mathews et al. 1973; Másdóttir et al. 2000; Sopori 2002). Smoking may also affect the balance of function between helper T-cell subsets 1 and 2 (Th1 and Th2), because researchers have observed increases in Th2- and/or Th1-related cytokines in smokers (Tsunoda et al. 2003; Cozen et al. 2004). In vitro experiments suggest that nicotine impairs the immunostimulatory activity of dendritic cells (antigen-presenting cells) and adversely affects the differentiation of monocytes into dendritic cells (Nouri-Shirazi and Guinet 2003; Guinet et al. 2004). Finally, studies have also associated smoking with low counts and reduced cytotoxic activity of natural killer cells, which are important components of innate immunity (Tollerud et al. 1989; Zeidel et al. 2002). Potential mechanisms through which exposure to tobacco or nicotine might result in an altered immune function include the induction of glucocorticoid hypersecretion and the increased release of catecholamines, which both inhibit the immune response or the activation of the autonomic nervous system (Sopori and Kozak 1998; Borovikova et al. 2000; Sopori 2002). Activation of the parasympathetic arm of the autonomic nervous system attenuates the systemic inflammatory response.

Studies suggest that smoking also induces systemic chronic inflammatory effects, which is possibly a consequence of increased oxidative stress (Cross et al. 1999; Hecht 1999; van der Vaart et al. 2005). As described earlier in this section, smokers have a leukocytosis compared with white blood cell counts in nonsmokers, and studies have associated smoking with elevated levels of C-reactive protein (Tracy et al. 1997; Wong et al. 2001; Bermudez et al. 2002). However, findings in studies of cytokine profiles in blood are not consistent (van der Vaart et al. 2005). Some studies suggest that smoking suppresses the production of proinflammatory cytokines such as IL-1, IL-6, and TNFα, which are important components of the immune response to intracellular pathogens such as viruses and fungi (Sopori and Kozak 1998; Ouyang et al. 2000). However, other studies have shown an enhanced production of IL-6 and TNFα, as well as other cytokines including IL-1β (Zeidel et al. 2002; van der Vaart et al. 2005).

Smoking could contribute to an increased risk of adverse pregnancy outcomes by its effects on the immune system through an increased risk of maternal infection, an alteration of the inflammatory response, or both. For example, studies have consistently associated smoking with a twofold-to-threefold increase in risk for bacterial vaginosis (Morris et al. 2001), which is a risk factor for preterm delivery. Researchers have hypothesized that smoking increases this risk through its effects on vaginal flora or through the depletion of Langerhans cells, resulting in local immunosuppression (Smart et al. 2004). Smoking can also reduce zinc levels, which could increase susceptibility to vaginal infections (Edman et al. 1986; Sikorski et al. 1990; Shubert et al. 1992). Cigarette smoking has been associated with increased cervical anti-inflammatory cytokines in early pregnancy, which could make women who smoke more vulnerable to reproductive tract infections and subsequent preterm delivery (Simhan et al. 2005, 2009). Finally, the immunosuppressive effects of smoking could contribute to protective effects against preeclampsia, because preeclampsia appears to involve an exaggerated or inappropriate immune response. More research is needed to fully define these potential relationships and pathways.

Tobacco Smoke Toxicants and the Reproductive System

Carbon Monoxide

Toxicity

CO is formed as a by-product of combustion and is thus present in tobacco smoke. It is a potent and even lethal toxin whose primary target organ is the brain. The fetus is more susceptible to the toxic effects of CO than is the mother. Symptomatic exposures to CO that the mother will fully recover from may end in permanent neurologic damage to the fetus or even fetal death (e.g., stillbirth) (Norman and Halton 1990; Koren et al. 1991). The fetal effects of CO are well studied (Koren et al. 1991; Penney 1996).

CO is the toxin found in the highest concentration in cigarette smoke. The dose per cigarette is 10 to 20 times the dose of nicotine (Hoffman et al. 1997). Furthermore, CO is not found in unsmoked tobacco products. The toxic effects of CO result predominantly from its binding to hemoglobin (Longo 1976, 1977). Each molecule of hemoglobin can carry four molecules of O_2 (Hsia 1998). The binding and unbinding of O_2 to hemoglobin depends on the local level of O_2. High levels of O_2 facilitate binding to hemoglobin, and low levels (hypoxia) facilitate the release of O_2 from hemoglobin. The O_2 binds to hemoglobin as blood passes through the O_2-rich lungs and is delivered to tissues as blood traverses the capillary beds. When one molecule of O_2 is released from hemoglobin, a conformational change in hemoglobin facilitates the release of further O_2 molecules.

Hypoxia, Fetal Growth, and Other Abnormalities

CO binds to hemoglobin with an affinity more than 200 times that of O_2 (Sauter 1994). Once CO binds to one of the four binding sites of hemoglobin, the hemoglobin is altered so greater tissue hypoxia is required before O_2 will be released from the other binding sites (Hsia 1998). In addition, CO prevents the conformational change in hemoglobin that occurs with O_2 unbinding. The release of one O_2 molecule does not facilitate the release of subsequent O_2 molecules when hemoglobin also binds CO.

The binding of CO to hemoglobin is tenacious, with a half-life of five to six hours. Fetal hemoglobin binds CO more tightly than does adult hemoglobin, and the fetus has higher levels of carboxyhemoglobin than those of the mother; the average ratio of fetal to maternal carboxyhemoglobin is 1.8 (Cole et al. 1972; Longo 1977; Bureau et al. 1982). It takes approximately seven hours for CO to equilibrate between the mother and the fetus (Bureau et al. 1982). The net effect of the CO and hemoglobin interaction is chronic hypoxia in fetal tissue (Longo 1977) or, more accurately, chronic cellular hypoxia that persists during periods of maternal abstinence from smoking, such as during sleep. Simply put, CO from cigarette smoke deprives the fetus of O_2, which is essential for the aerobic metabolism that produces adenosine triphosphate (ATP). ATP stores chemical energy that is ubiquitously used to drive all manner of chemical reactions in the body. This chronic yet mild O_2 deprivation in the fetus is likely a major underlying mechanism of smoking-associated fetal growth retardation (Longo 1976, 1977).

Data from both clinical and animal studies indicate that CO is probably the foremost toxin responsible for the LBW associated with maternal smoking (Garvey and Longo 1978; Lynch and Bruce 1989; Penney 1996; England et al. 2003). A well-designed study found a decrease in birth weight that was almost five times greater for infants of smokers than for infants of snuff users, even after adjustment for variables (England et al. 2003). The mean adjusted decrease in birth weight was 39 g for infants of snuff users and 190 g for infants of smokers compared with infants of nonsmokers. Because CO is the main toxin in cigarette smoke but not in snuff, this difference in birth weight implicates CO as the likely hazard. Even mild, long-term exposure to CO in animals resulted in fetal growth retardation; maternal carboxyhemoglobin levels were 4 to 9 percent (Garvey and Longo 1978; Penney 1996). The carboxyhemoglobin levels associated with smoking are 5 to 10 percent.

Studies have found central nervous system abnormalities in fetuses and pups of pregnant rats with long-term exposure to CO (Storm and Fechter 1985a,b; Storm et al. 1986; Fechter 1987; Carratù et al. 1993a,b; Packianathan et al. 1993). Behavioral studies of prenatally exposed animals have revealed persistent postnatal effects associated with CO exposure that produced maternal carboxyhemoglobin levels of 6 to 16 percent. These levels were not associated with small litter size or altered duration of gestation but with LBW (Fechter and Annau 1976, 1980, 1997; Abbatiello and Mohrmann 1979; Mactutus and Fechter 1984, 1985; Singh 1986; Fechter 1987). CO-induced hypoxia appears related to other congenital anomalies including cleft lip and cleft palate in susceptible strains of mice (Millicovsky and Johnston 1981; Bronsky et al. 1986; Bailey et al. 1995). Subsequent epidemiologic studies of birth defects in relation to CO levels from air pollution early in gestation found associations between higher CO levels and various cardiac defects, but the findings were not consistent (Ritz et al. 2002; Gilboa et al. 2005).

Blood Hyperviscosity

Carboxyhemoglobin results in functional anemia in both the mother and fetus that stimulates production of red blood cells and elevates maternal and fetal hematocrits (Meberg et al. 1979; Bureau et al. 1983; Bili et al. 1996). As the hematocrit increases, the viscosity of the blood increases. At birth, the healthy newborn hematocrit is normally 44 to 64 percent, which is well above adult values. Because of the increased viscosity, healthy newborns are at risk for stroke if the hematocrit is above 65 percent. The hematocrit can be lowered with partial-exchange transfusions. Compared with newborns of nonsmokers, newborns of smokers have higher hematocrits that therefore increase the risk of stroke and a need for exchange transfusion (D'Souza et al. 1978; Buchan 1983). Elevated maternal hematocrits and a consequently higher blood viscosity in the mother may also be risk factors for suboptimal placental perfusion (Bureau et al. 1983; Knottnerus et al 1990).

Preeclampsia

Data indicate that CO functions as a gaseous localized messenger (Ryter et al. 2004). CO appears to activate guanylate cyclase and modulate the mitogen-activated protein kinase signaling pathway (Ryter et al. 2004). Heme oxygenases (HO-1, HO-2, and HO-3) degrade heme into ferrous ion, CO, and biliverdin, which all have important physiological functions at low concentrations but are toxic in high concentrations (Ryter et al. 2004). CO appears to have localized functions similar to those of nitric oxide (NO), a gas that affects vascular tone and platelet aggregation (Ryter et al. 2004). CO also appears to have cytoprotective effects against oxidative stress by

reducing inflammation and suppressing apoptosis (Ryter et al. 2004; Tsuchihashi et al. 2004).

Researchers think it is likely that the CO from cigarette smoke is responsible for the reduced risk of preeclampsia associated with smoking. The basis for this rationale is that users of snuff, which does not contain CO, have an increased risk of preeclampsia (England et al. 2003). The pathophysiology of preeclampsia remains to be elucidated, but the transformation of the spiral arterioles, which supply blood to the placenta, into low-resistance high-flow vessels appears to be incomplete (see "Preeclampsia" and "Placenta" earlier in this chapter). Spiral arteries may still be responsive to vasoconstrictive stimuli. Episodic constriction resulting in reduced blood flow to the placenta can cause hypoxia-reperfusion injury, which elicits endothelial damage and an inflammatory response. A hypoxic uterine environment appears to be a normal stimulus for the transformation of spiral arterioles during pregnancy (Lyall 2003). CO via carboxyhemoglobin can augment this tissue hypoxia and may stimulate the normal transformation of spiral arterioles. Additionally, CO functions similarly to NO as a vasorelaxant and may counteract the effects of circulating vasoconstrictive agents on preeclamptic spiral arterioles. Both hypoxic environment and vasorelaxation may help to prevent hypoxia-reperfusion injury and the consequential inflammation and endothelial damage, thereby reducing the risk of preeclampsia (Bainbridge et al. 2005).

Independent of preventing hypoxia-reperfusion injury, CO may function similarly to NO in maintaining normal endothelial function and preventing platelet aggregation. Also, research in the area of tissue transplantation has found that exogenous CO significantly reduces the inflammatory environment in allograft rejections (Tsuchihashi et al. 2004). Researchers postulate that CO may have a similar role in the heightened inflammatory environment of the preeclamptic placenta. Although some evidence supports this model of CO and preeclampsia, the data are not extensive (Barber et al. 2001; McLaughlin et al. 2001). Investigators do not know how important CO is as a local messenger during pregnancy or how exogenous CO supplements endogenous CO.

Summary

CO is the toxin in cigarette smoke that is found in the highest concentrations. The major effect of CO is to deprive the fetus of O_2 by binding to hemoglobin. The binding of CO to hemoglobin also results in functional anemia that eventually produces a rise in the hematocrit. Elevated hematocrit in the mother may adversely affect blood flow in the placenta, leading to placental problems and potentially to fetal growth retardation. CO appears to prevent preeclampsia by augmenting uterine hypoxia and, thus, development of arterioles or other local effects similar to those of NO. However, this possibly beneficial role for CO is far outweighed by its hypoxic effects involving hemoglobin.

Nicotine

Nicotine, the principal alkaloid in tobacco, is a major contributor to the addictive properties of smoking. The diverse pharmacologic and toxicologic properties of nicotine are discussed in Chapter 4, "Nicotine Addiction: Past and Present," and are only briefly touched on here. Nicotine has both short- and long-term effects and is likely causally related to several of the endpoints discussed in this chapter. The Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency (Cal/EPA) lists nicotine as a developmental toxicant. Nicotine is known to cross the placenta and concentrate in the fetus at levels slightly higher than those in the mother. Nicotine may decrease placental perfusion, leading to hypoxia of the fetus and acidosis.

As noted earlier in this chapter, nicotine may be involved in the development of various congenital anomalies or neurobehavioral problems. Experimental studies of rhesus monkeys exposed to nicotine in utero showed decreases in fetal lung weight, volume, and function (Sekhon et al. 2001) similar to those observed in offspring of maternal smokers (see "Fetal Tissue and Organogenesis" earlier in this chapter). Thus, nicotine may be the key constituent of tobacco smoke to impair fetal lung development and lead to altered lung function and perhaps increased respiratory illness. In an experiment with chick embryos, low doses of nicotine induced hyperactivity and higher doses induced hypoactivity (Ejaz et al. 2005). The researchers concluded that nicotine could alter embryonic movements that are important during embryogenesis for the differentiation and maturation of the embryo's organ systems. McCartney and colleagues (1994) speculated that intrauterine exposure to nicotine specifically affects the outer hair cells in the ear, which influence language ability, leading to poorer performance scores on assessments that rely heavily on verbal abilities.

Nicotine may also interfere with pregnancy by affecting oviduct function, which may lead to ectopic pregnancy or problems with fertilization and implantation, or by affecting transport of essential nutrients, which could affect fetal growth (see earlier sections). For example, nicotine altered oviduct motility in rhesus monkeys (Neri and Marcus 1972), decreased oviductal blood flow in rats (Mitchell and Hammer 1985), and decreased sodium and potassium concentrations in oviductal epithelial cells of mice (Jin et al. 1998). In vitro studies report

that nicotine, CO, and cyanide impair amino acid uptake in placental microvilli (Rowell and Sastry 1978; Horst and Sastry 1988; Sastry 1991). In addition, nicotine may impair amino acid transport (Fisher et al. 1984). Studies show reduced levels of several amino acids in fetal plasma, umbilical plasma, and placental villi in maternal smokers compared with those in nonsmokers (Jauniaux et al. 1999, 2001), and nicotine inhibits in vitro transport of arginine in human placentas (Pastrakuljic et al. 2000) (see "Amino Acids" later in this chapter). The reports of associations of smokeless tobacco use with several adverse pregnancy outcomes, such as LBW, preterm delivery, stillbirth, and placental morphologic changes (Agrawal et al. 1983; Gupta and Sreevidya 2004; Gupta and Subramoney 2006), suggest that a component of tobacco smoke in addition to CO, perhaps nicotine, contributes to these toxic effects.

Nicotine appears to be one of the components of tobacco smoke that has endocrine-disrupting effects, which, in turn, may affect several other reproductive and developmental endpoints. In vitro experiments show that treatment of cells with alkaloids found in tobacco smoke (namely, nicotine, cotinine, anabasine, or a combination of these substances) or with an aqueous extract of cigarette smoke resulted in a dose-dependent inhibition of progesterone production (Bódis et al. 1997; Gocze et al. 1999; Gocze and Freeman 2000; Miceli et al. 2005), whereas estradiol production showed little effect or was slightly stimulated. These findings support the effects of smoking on progesterone observed in epidemiologic studies. Cell growth and DNA content also decreased with treatment, leading the authors to suggest that smoking directly inhibits cellular progesterone synthesis through less specific cytotoxic effects on progesterone-producing cells (Gocze and Freeman 2000). Other scientists concluded that nicotine and M-nicotine, the methylated metabolite, can induce a type of luteal insufficiency by inhibiting progesterone release, probably through modulations in the prostaglandin system (Miceli et al. 2005) or inhibition of aromatase enzymes.

In animal models, nicotine acts on the HPG axis to increase secretion of adrenocorticotropic hormone from the pituitary gland, which then stimulates production of adrenocortical hormone (Matta et al. 1998). This finding is consistent with hormone profiles observed in clinical studies (see "Endocrine System" earlier in this chapter). Studies have also reported that nicotine acts directly on steroidogenesis by inhibiting various hydroxylases involved in their metabolism and on aromatases involved in converting androgens to estrogens (Barbieri et al. 1986a,b, 1987). Animal studies show that prenatal exposure to nicotine is related to decreased testosterone levels in adult male rats (Segarra and Strand 1989). They also report that cotinine, but not nicotine, inhibits testosterone synthesis in testes of neonatal rats (Sarasin et al. 2003). A small study of administration of nicotine to men and women by a transdermal patch found that the patch significantly lengthened the interpulse interval of pulsatile LH secretion in male nonsmokers but not in female nonsmokers or in smokers (Funabashi et al. 2005).

Metals

Presence in Tobacco Smoke

The particulate component of tobacco smoke contains metals. Their presence depends on the origin of the tobacco, the formulation of the cigarette product, and the method of smoking; detection depends on the method and sensitivity of the analysis. Analyses have quantified cadmium (<1.2 to 90.3 nanograms [ng] per cigarette), lead (0 to 41.4 ng/microgram [µg]), and mercury (<0.25 to 4.3 ng/µg) in mainstream smoke (Houlgate 2003). Nickel and chromium were not detectable (limit of detection = 1.8 and 1.7 ng per cigarette, respectively), although other studies have identified these metals in tobacco smoke (Smith et al. 1997; Torjussen et al. 2003). Arsenic was detectable but not quantifiable (limit of quantitation = 2.7 ng per cigarette). Studies have also detected additional metals such as zinc (U.S. Environmental Protection Agency [USEPA] 1992) and beryllium (Smith et al. 1997) in cigarette smoke. In sidestream smoke, there are estimated amounts only for cadmium, nickel, and zinc (National Research Council 1986). Very few animal toxicology studies of metals, which are reviewed here, used inhalation exposure. Clinical data are available on the bioavailability of cadmium and nickel from cigarette smoke, but researchers have studied only cadmium in connection with smoking-induced toxic effects on reproduction and development.

Exposure of reproductive organs to metals from cigarette smoke depends on (1) the uptake from the lung to the circulation, (2) the presence of transporters at blood-tissue barriers, and (3) the regulation of uptake and egress at the cellular level. In the fetus and the testes, there are physical barriers to blood flow (the placenta and the blood-testes barrier) and highly selective metal transport mechanisms (Hidiroglou and Knipfel 1984; Sylvester and Griswold 1994; Ballatori 2002; Asano et al. 2004; Gruper et al. 2005).

General Mechanisms

Metals reaching the cells and reproductive organs of the fetus can act through several common mechanisms. Transition metals, which can assume more than one

valence state, can influence electron-exchange reactions and oxidative stress within cells. Metals can also substitute for the appropriate trace element at sites where nutritionally essential trace elements are important, such as active sites of enzymes, sites for regulatory elements of transcription factors, and metal-binding sites in receptor complexes or ion channels. Metals can also displace essential trace elements at storage sites, such as the bone matrix or heme molecules. Most mechanisms are posited for metals in the ionic form. Various compounds that incorporate metals may take metals from cigarette smoke before reaching the circulatory system and reproductive organs. However, the relevance of the various biologic actions of metals in smoking-related reproductive disease awaits further research. Current assessments must rely on parallels between smoking-related and metal-induced adverse reproductive effects.

Reproductive Effects of Specific Metals

The toxic effects of the heavy metals lead, mercury, and cadmium on reproduction and development are well known and widely reviewed in both clinical and animal studies (Clarkson et al. 1985; Andrews et al. 1994; Goyer and Clarkson 2001). The toxicity of mercury and lead is highly dependent on whether the metal is organic or inorganic. The most sensitive endpoint for lead and methyl mercury is the neurobehavior of children (Mendola et al. 2002). In addition, male and female reproductive effects from metal toxicity are well documented, including effects on fertility, menstrual cycle function, and adverse pregnancy outcomes (Ward et al. 1987; Golub 2005b; Hoyer 2005; Sokol 2005). At low levels of exposure that are potentially relevant to cigarette smoke, studies have not demonstrated effects on fertility in women but have associated infertility with paternal occupational exposure to lead (Sallmén et al. 2000). Literature reviews have indicated associations between prenatal exposure to lead and SAB, preterm delivery, and reduced birth weight (Andrews et al. 1994; Antilla and Sallmén 1995; Borja-Aburto et al. 1999). One study associated exposure to lead with delayed puberty (Selevan et al. 2003), but exposure to mercury had little effect on the timing of puberty (Denham et al. 2005).

An extensive number of studies on exposure to lead in male animals all report abnormalities in spermatogenesis and production of reproductive hormones. Studies of men report an inverse relationship between levels of lead in blood and levels in sperm, in addition to adverse pregnancy outcomes in their partners (Anttila and Sallmén 1995; Lin et al. 1998; Sokol 2005). The use of mercury in dental amalgams has led to studies of dentists and dental assistants, but evidence for reproductive effects in either males or females is limited. One study found decreased fertility in female dental assistants with greater exposure to lead (Rowland et al. 1994). Sperm production is affected in animal models with exposure to some mercury doses. Finally, experimental studies of exposure to mercury in birds and fish demonstrate hormonal effects relevant to endocrine disruption (Golub 2005b).

Researchers have investigated cadmium as the agent in cigarette smoke responsible for LBW in newborns of smokers. Studies document that cadmium accumulation in the blood and placentas of pregnant smokers correlated with LBW (Kuhnert et al. 1982, 1987a). Other studies associated placental cadmium, but not blood cadmium, with LBW of newborns of smokers (Ward et al. 1987; Sikorski et al. 1988). Studies have reported inconsistent associations between exposure to cadmium and birth weight in newborns of women exposed to cigarette smoke in the workplace or by environmental contamination (Huel et al. 1981, 1984; Bonithon-Kopp et al. 1986; Berlin et al. 1992; Loiacono et al. 1992; Fréry et al. 1993; Nishijo et al. 2002). LBW was a significant finding in some studies that administered cadmium, usually as cadmium chloride, to rats and mice by injection, inhalation, or orally in food and drinking water (Cal/EPA 1996). Many of these studies found delayed ossification, another indicator of developmental delay. At higher doses, fetal viability was affected.

One proposed mechanism of the effect of cadmium on birth weight is interference with the placental transfer of the essential trace elements zinc and copper (Sowa et al. 1982; Steibert et al. 1984; Sasser et al. 1985; Sowa and Steibert 1985; Kuhnert et al. 1987a; Chmielnicka and Sowa 1996). Researchers hypothesize that cadmium also interferes with progesterone production in the placenta (Jolibois et al. 1999a,b; Piasek et al. 2001; Kawai et al. 2002; Henson and Chedrese 2004). Studies have found that cadmium acts as an estrogenic agent. Initially, in vitro studies demonstrated that cadmium binds to a specific site on the estrogen receptor and mimics estradiol-induced gene transcription (Garcia-Morales et al. 1994; Choe et al. 2003; Johnson et al. 2003b). Other studies found that the effects of in vivo administration of cadmium on the uterus and mammary glands could be blocked by antiestrogenic agents (Johnson et al. 2003b). Animal studies show that cadmium accumulates in ovaries, that there is a loss of ovarian follicles, and that steroid production declines (Hoyer 2005). Elevated levels of cadmium in the follicular fluid of smokers (Zenzes et al. 1995) were not associated with impaired fertility (Drbohlav et al. 1998; Younglai et al. 2002). Studies have also associated smoking with elevated cadmium levels in seminal fluid. At least one study noted a negative correlation with cadmium levels

and semen quality, but another found no correlation (Saaranen et al. 1989; Chia et al. 1994). Animal studies have shown some negative effects on spermatogenesis.

Chromium, nickel, and zinc are essential human dietary nutrients (Institute of Medicine 2000). They are present in tobacco and have been studied for their toxic effects on reproduction and development (Keen 1996; Golub 2005a). Almost all information on toxicity comes from laboratory animal studies, and very little is known about exposure through inhalation. Exposure to chromium (as Cr^{+6}) produced embryo and fetal loss, growth restrictions, and malformations when administered in drinking water to mice at a minimum dose of 60 mg/kg per day (Trivedi et al. 1989; Junaid et al. 1995, 1996). Studies show that chromium is a testicular and ovarian toxicant that also affects fertility when administered in drinking water to rodents (Saxena et al. 1990; Zahid et al. 1990; Murthy et al. 1991, 1996; Bataineh et al. 1997). Nickel is teratogenic in mice and rats when it is injected (Lu et al. 1979; Mas et al. 1985). When nickel was administered over a long period in drinking water, perinatal mortality was a common finding (Smith et al. 1993). Studies have also demonstrated the testicular toxicity of nickel that was injected intraperitoneally or administered orally to rodents, but ovarian toxicity and male fertility were not studied (Kakela et al. 1999; Doreswamy et al. 2004). Long-term studies that administered zinc to male and female rodents found no effects on fertility (Ogden et al. 2002; Johnson et al. 2003a).

Growing evidence suggests adverse effects on human pregnancy outcomes (e.g., stillbirth, SAB, and LBW) from exposure to arsenic in drinking water (Hopenhayn-Rich et al. 2000; Ahmad et al. 2001; Hopenhayn et al. 2003; Yang et al. 2003). Animal studies demonstrate toxic effects on ovaries and testicles from arsenic in drinking water (Chattopadhyay et al. 1999, 2001; Pant et al. 2001), and earlier literature discusses arsenic teratogenesis (Golub et al. 1998). These studies support further efforts to assess the bioavailability of arsenic from cigarette smoke.

Most of these metals, such as lead, cadmium, mercury and mercury compounds, nickel carbonyl, and inorganic oxides of arsenic, are listed as "known by the state to cause reproductive toxicity" under California's Proposition 65 program, affecting a variety of endpoints. (Information supporting the listings can be found at the agency's web site [http://www.oehha.ca.gov].) Thus, some or all of these compounds may contribute to the adverse effects of smoking on reproduction, but direct links in smokers have not been established.

Polycyclic Aromatic Hydrocarbons

Formation and Toxicity

PAHs are ubiquitous products of the partial combustion of carbon-containing materials, and they appear as important components of environmental pollution. Although some sources are natural, the predominant sources of PAHs found in the air are usually anthropogenic. Examples include vehicle exhausts, products from industrial processes, and emissions from fossil fuel power plants (International Agency for Research on Cancer [IARC] 1983), as well as tobacco smoke (IARC 1986, 2004; USEPA 1992). The usual definition of a PAH specifies hydrocarbons with no heteroatom substitutents or ring members that include at least two or, according to some authors, three concatenated aromatic (usually benzene-like) rings. The two-ring members of the class, primarily naphthalenes, are included within the definition used in EPA's identification of "polycyclic organic material" as a hazardous air pollutant. These two-ring members are abundant in tobacco smoke and show some chemical and toxicologic differences from other PAHs. This discussion primarily addresses the effects of PAHs with three or more rings, while also noting some specific effects of naphthalenes. The five-ring compound benzo[*a*]pyrene (B[*a*]P) is one of the most extensively studied PAHs. In addition to carcinogenesis, studies have reported direct fetotoxic and teratogenic effects associated with PAHs, as well as adverse effects on reproduction. Other notable effects include immunotoxicity, endocrine effects, and toxic effects on the lungs. Key studies are summarized in Table 8.12, and the results are discussed in detail here.

The toxic effects and dose-response relationships described for specific PAHs are primarily based on experiments on toxic effects in animals, which are the focus of this summary. Several corresponding effects in humans result from exposure to pollutant mixtures containing PAHs, such as diesel exhaust. Human exposure to PAHs generally involves mixtures that are ill defined and poorly quantified, so it is difficult to separate the effects of PAHs from those of other components of the mixtures.

Most of the toxic endpoints described for PAHs appear to result from the generation of reactive intermediate agents by metabolism, followed by reactions of these intermediates (e.g., as B[*a*]P 7,8,9,10-dihydrodiol) with the cellular components, particularly DNA, in both adult and fetal tissues (Kleihues et al. 1980; Bolognesi et al. 1985; Shugart and Matsunami 1985). Unless repaired, the adducts that are produced give rise to mutations that

Table 8.12 Reproductive and developmental effects of polycyclic aromatic hydrocarbons (PAHs), by endpoint

Study	Design: animal model or population	Endpoint
Krarup 1969	Direct application of 9:0-dimethyl-1:2-benzanthrene to mouse ovary	Toxic effects to reproductive system in adults
Mattison and Thorgeirsson 1979	PAH treatment (80 mg/kg) of juvenile mice (4–6 weeks old): C57BL/6N (AHH inducible) and DBA/2N (AHH noninducible)	Toxic effects to reproductive system in adults
Shum et al. 1979	C57BL/6 (AHH responsive) or AKR (nonresponsive) mice exposed to B[*a*]P (50–300 mg/kg intraperitoneally) at day 7, 10, or 12 of gestation	Teratogenicity
Mackenzie and Angevine 1981	Mice exposed prenatally to B[*a*]P (10, 40, or 160 mg/kg per day) on days 7–16 of gestation Fertility study Beginning at 7 weeks of age, each F_1 male was placed with 10 untreated females for 25 days Beginning at 6 weeks of age, each F_1 female was cohabitated continuously with an untreated male for 6 months Fertility index = females pregnant/females exposed to males x 100	Birth weight Adult reproductive function after prenatal exposure
Mattison and Nightingale 1982	Mice (AHH responsive or nonresponsive) exposed to B[*a*]P (100 mg/kg intraperitoneally)	Toxic effects to reproductive system in adults
Urso and Gengozian 1982; Urso and Johnson 1988; Urso et al. 1992	Series of experiments in mice: single exposure to B[*a*]P during pregnancy (150 mg/kg intraperitoneally)	Developmental immunotoxicity Immune response measure: degree of anti–sheep erythrocyte plaque-forming response, mixed lymphocyte response of cultured lymphocytes, measures of T-cell function

Findings	Comments
• Oocyte destruction and ovarian tumorigenesis	A typical effect reported early, although the route of application is artificial
• Number of primordial oocytes severely depleted in ovaries of mice exposed to carcinogenic PAHs (B[*a*]P, 3-methylcholanthrene, 7,12-dimethylbenz[*a*]anthracene, but not by a noncarcinogenic PAH or an AHH inducer (pyrene, β-naphthoflavone) • PAHs eventually resulted in complete destruction of oocytes in both strains, but effect is faster in AHH inducible strain	Destruction of oocytes is related to inducibility of AHH; researchers assumed in this case that activation of the enzyme led to generation of more reactive metabolites, which caused greater oocyte destruction: this is supported by the inactivity of β-naphthoflavone, an AHH inducer not metabolized to reactive intermediates; however, other AH receptor actions may also be involved
• In utero toxicity and teratogenicity: stillbirths, resorptions, malformations increased in exposed mice • Effects approximately 2–3 times as severe in C57BL/6 mice, which are responsive to AHH induction at all exposure times • B[*a*]P (200 mg/kg intraperitoneally)	
• Pup weights significantly reduced compared with those for controls at all doses ($p < 0.01$) at 20 and 42 days; decreasing trend in pup weight with dose at day 4 • Exposed mice of both sexes as adults showed loss of fertility in controlled breeding studies with untreated partners • Mice given B[*a*]P at 40 or 160 mg/kg per day essentially infertile ($p < 0.01$) with histologic abnormalities of gonads • Testes of males exposed to B[*a*]P at 40 mg/kg showed severe atrophy and aspermic seminiferous tubules • Ovaries of exposed females hypoplastic or atrophied with few follicles or corpora lutea • Mean litter size reduced ($p < 0.01$) with treatment of females but not with treatment of males	
• 30% oocyte destruction of primordial oocytes	Effect was not linked to AHH induction in this experiment
• Increases in Lyt2 cells in fetal liver • Often profound immunosuppression in neonatal offspring and later in life; postnatal thymic and splenic suppression • Approximately 50% reduction in plaque-forming cells in sheep erythrocyte assay in 1-week-old offspring after mid- or late-gestational treatment with 150 µg of B[*a*]P • Changes in maternal immune system, which may affect maintenance of pregnancy: thymic suppression, including reduction in several classes of T lymphocytes and inhibition of mixed lymphocyte response	

Table 8.12 Continued

Study	Design: animal model or population	Endpoint
Holladay and Smith 1994	B6C3F$_1$ mice exposed to B[*a*]P (maternal dose of 50, 100, or 150 mg/kg/day) on days 13–17 of gestation	Developmental immunotoxicity Offspring examined for T-cell and fetal liver cell markers on gestational day 18
Lummus and Henningsen 1995	BALB/c mice exposed to B[*a*]P in utero: single dose of 150 mg/kg of B[*a*]P on days 11–13 of gestation	Developmental immunotoxicity
Nicol et al. 1995	Exposure to B[*a*]P in utero Normal mice and mice deficient in the *P53* tumor suppressor gene	Teratogenicity
Perera et al. 1998	Epidemiologic exposure to PAHs by ambient air pollution in Poland 70 newborns from Krakow (industrialized) and 90 from Limanowa (rural but coal used for home heating) PAH-DNA adducts in leukocytes and plasma cotinine measured in umbilical cord blood	Birth weight and developmental delay

Findings	Comments
• Severe depletion of T cells in both thymus and liver • Changes in proportions of surface antigens (CD4, CD8, and heat stable) in isolated thymocytes • Reduction in total cellularity of thymus and liver	Researchers suggested that these changes indicated impaired maturation in surviving thymocytes consistent with long-term immunosuppression seen in mice exposed to B[*a*]P in utero
• Numbers of T cells in fetal liver severely reduced compared with those for untreated controls; severe atrophy of fetal thymus • Neonates showed decreased overall thymic cells and increases in some splenic cells • 6-week-old juvenile mice showed recovery of total T cells to control levels in spleen and thymus but depletion of thymic cells bearing fetal liver T-cell antigen	Changes in relative proportions of different types of T cells, distinguished by various surface antigens, result in disturbance of immune system development; some apparently B[*a*]P-resistant cell types repopulate depleted lymphoid organs, but overall function of the immune system is changed and remains depressed
• Malformations and increased rate of fetal death • Embryotoxicity and teratogenicity 2- to 4-fold higher in *P53*-deficient mice than in controls	The *P53* gene, which is important in regulation of DNA repair and apoptosis, has a significant embryo-protective effect in the B[*a*]P-exposed fetus
• In newborns with high (>median) versus low (≤median) levels of PAH-DNA adducts: – Mean birth weight reduced by 147 g ($p < 0.05$) – Birth length reduced by 1.1 cm ($p < 0.02$) – Head circumference reduced by 0.9 cm ($p < 0.001$) • Cotinine also significantly and inversely associated with low birth weight and reduced length	

Table 8.12 Continued

Study	Design: animal model or population	Endpoint
Dejmek et al. 2000	Epidemiologic exposure to PAHs and PM by ambient pollution in Czech Republic 3,349 births in Teplice and 1,505 in Prachatice between April 1994 and March 1998 Continuous measurement of PAH, PM_{10}, and $PM_{2.5}$ AOR for IUGR correlated monthly with PAH and PM pollution levels	Birth weight and developmental delay
Matikainen et al. 2001	Young mice, oocytes, and human ovarian tissue explants exposed to 7,12-dimethylbenz[*a*]anthracene	Toxic effects to reproductive system in adults

Note: **AH** = aryl hydrocarbon; **AHH** = aryl hydrocarbon hydroxylase; **AOR** = adjusted odds ratio; **B[*a*]P** = benzo[*a*]pyrene; **CI** = confidence interval; **cm** = centimeters; **g** = grams; **IUGR** = intrauterine growth retardation, where birth weight is <10th percentile by gender and gestational week; **mg/kg** = milligrams per kilogram; **ng** = nanograms; **ORs** = odds ratios; **PM** = particulate material; $\mathbf{PM_{10}}$ = particulate matter in which particles are ≤10 micrometers; $\mathbf{PM_{2.5}}$ = particulate matter in which particles are ≤2.5 micrometers; **µg** = micrograms.

Findings	Comments
• AORs for IUGR show significant increases with levels of PAH at both locations early in pregnancy during first month of gestation; AOR for medium exposure = 1.63 (95% CI = 0.87–3.06) and for high exposure, 2.39) (95% CI = 1.01–5.65) • In Prachatice, relationship was more noticeable (AOR 2.44 [95% CI, 0.6–9.83]) in subset closer to pollutant source • Pollution in Teplice high in both PM and PAH, but in Prachatice, high in PAH and generally lower in PM • IUGR was observed in 9.6% of pregnancies in Teplice and 8.2% in Prachatice	Because the ratio of PM and PAH varied between locations and with time, the continuous model was able to show that the risk of IUGR increased with level of PAH exposure, rather than with PM, and resulted from an early developmental defect

Risk of IUGR with level of exposure to pollutant in first month

Pollutant/ location	Comparison by exposure	AOR	95% CI
PM_{10}			
Teplice	Medium : low	1.44	1.03–2.02
	High : low	2.14	1.42–3.23
Prachatice	Medium : low	2.11	1.03–4.33
	High : low	1.09	0.49–2.46
PAH			
Teplice	Medium : low	1.59	1.06–2.39
	High : low	2.15	1.27–3.63
Prachatice	Medium : low	1.49	0.81–2.73
	High : low	1.26	0.60–2.63

Increase in AOR for IUGR for each 10 µg of PM_{10} or 10 ng of PAH during first month*

Pollutant/ location	AOR	95% CI
PM_{10}		
Teplice	1.19	1.06–1.33
Prachatice	1.04	0.86–1.27
PAH		
Teplice	1.22	1.07–1.39
Prachatice	1.17	0.92–1.89

*ORs adjusted for parity, maternal age and height, prepregnancy weight, education, marital status, season, year of study, and maternal smoking per month

• Oocyte destruction and ovarian failure; mechanism involves enhancement of apoptosis	Effect is dependent on the *BAX* promoter gene and the AH receptor

are then followed by cytotoxicity and/or cancer and possibly teratogenicity (Wells and Winn 1996). Both phase I (activation) enzymes and phase II (detoxification and conjugation) enzymes are important in the metabolism and toxicity of PAHs, and both are inducible by PAHs. The structural genes determining the stability and activity of the enzymes and the regulatory genes controlling the expression of the enzymes, show polymorphisms in both humans and animals. One of these enzymes is aryl hydrocarbon hydroxylase (AHH), which is influenced by induction of cytochrome P-450 activity. Animals are described as genetically "responsive" when AHH activity is induced by exposure to PAHs and to other activators of the AH receptor. There are also important changes in the levels and types of enzymes expressed at different developmental stages, particularly during the latter part of fetal development and the immediate postnatal period (Cresteil et al. 1986). Researchers have used the resulting variations in metabolic capabilities of the fetus and young animal to investigate the mechanisms of and differential susceptibility to PAH toxicity.

Toxic Effects on Reproduction

Investigators have known for some time that exposure of the adult female rodent to PAHs damages the resting ovarian follicle complexes, leading to oocyte destruction (Krarup 1969; Mattison and Thorgeirsson 1979; Mattison and Nightingale 1982) (Table 8.12). Studies have revealed similar effects in women, primarily as premature reproductive senescence (menopause), after exposure to mixed pollutants. As noted previously, premature senescence is associated with smoking (Jick et al. 1977; USDHHS 1980) (see "Menstrual Function, Menarche, and Menopause" earlier in this chapter).

Mattison and Nightingale (1982) reported a 30-percent destruction of primordial oocytes in adult mice exposed to a single dose of B[*a*]P. After comparing susceptibility to this effect in different strains of mice that were responsive or unresponsive to inducers of cytochrome P-450 enzymes, these researchers suggested that the determining factor for oocyte destruction involves the ratio of phase II (detoxifying) to phase I (activating) enzyme activities. Later investigations have shown the involvement of mechanisms that control apoptosis in oocyte destruction (e.g., Matikainen et al. 2001). The researchers described three sets of studies using treatment with 7,12-dimethylbenz[*a*]anthracene in young mice, isolated oocytes, or xenografts of human ovary tissue. They found that both a functional AH receptor and a functional *BAX* promoter gene were necessary for oocyte destruction in mice. Moreover, this apoptosis control system was induced in oocytes by the activation of the aryl-hydrocarbon-responsive AH receptor. This finding provides an alternative direct route for triggering the cytotoxic response to PAHs, which is in contrast to the earlier proposal involving reactive PAH metabolites.

Toxic Effects on Development

Teratogenicity. Many fetotoxins, including PAHs, produce a spectrum of effects: anatomic and functional teratogenesis; prenatal, perinatal, and postnatal mortality; growth retardation; and developmental delay. To observe the combination of these outcomes in a particular experiment may depend on dose level and timing, the test species used, and other experimental conditions. The most commonly observed effects of PAHs in animal studies are growth retardation and fetal mortality, but a few experiments have demonstrated anatomic teratogenic effects. The number of surviving offspring is reduced in these experiments, so it appears that the dose range over which surviving, but malformed, offspring are produced is narrow.

Intraperitoneal B[*a*]P given to mice at day 7 or 10 of gestation causes toxic effects in utero (e.g., a reduction in the number of surviving offspring) and teratogenicity (Table 8.12) (Shum et al. 1979). The severity of the effect was correlated with the ability of the fetus and the maternal systems to metabolize B[*a*]P. A greater impact on prenatal and postnatal mortality was noted in C57BL/6 mice, which are responsive to induction of AHH, than in unresponsive AKR inbred mice. This finding suggests a role for reactive intermediate agents of PAHs. Malformations observed only in the responsive mice included clubfoot, hemangioendothelioma, cleft palate, and other anomalies of the skeleton and soft tissues.

Nicol and colleagues (1995) also observed malformations and an increased rate of fetal death after in utero exposure to B[*a*]P. The embryotoxicity and teratogenicity were twofold-to-fourfold higher in mice deficient in the *P53* tumor-suppressor gene than in the controls with the normal *P53* gene. The *P53* gene, which is important in the regulation of DNA repair and apoptosis, thus has a significant embryoprotective effect in the fetus exposed to B[*a*]P, which is also characteristic in relation to other DNA-damaging teratogens such as phenytoin (Nicol et al. 1995; Wells and Winn 1996).

Prenatal impacts on adult reproductive function. Animal studies have demonstrated similar but more drastic reproductive effects in both males and females exposed to PAHs in utero rather than as adults. As adults, offspring exposed to B[*a*]P in utero showed a loss of fertility in controlled breeding studies with untreated partners (Table 8.12). High doses of B[*a*]P resulted in complete infertility and histologic abnormalities of the gonads

(Mackenzie and Angevine 1981). Although most observations of reduced fertility in adults focused on females, this experiment also showed a clear reduction in fertility among the treated F_1 males. Examination of the testes showed severely atrophied and essentially aspermic seminiferous tubules. The ovaries of females were hypoplastic and had very few follicles or corpora lutea. Most of the animals exposed to the high doses had no identifiable ovaries or only remnants of ovarian tissue.

Kristensen and colleagues (1995) also reported reductions in fertility among female NMRI mice after exposure in utero to 10 mg of B[*a*]P/kg per day given orally. Watanabe (2005) reported decreases in the number of spermatozoa and Sertoli cells in the testes of adult rats exposed in utero to diesel exhaust.

Investigations of the mechanism of oocyte depletion have emphasized the importance of the AH receptor and *BAX* activation in the induction of apoptotic destruction of oocytes and in the natural process that reduces the initial fetal complement of primordial oocytes early in their development to the lower levels that characterize the adult female. These findings do not necessarily exclude a separate role for cytotoxic effects from DNA damage by reactive PAH metabolites in the destruction of germ cells. The mechanisms involved in the induction of impaired sperm quality and male infertility after adult or fetal exposure to PAHs are less extensively studied. Therefore, it is unclear whether other factors and/or mechanisms apply.

Effects on birth weight and developmental delay. Clinical studies of exposure to PAH-containing mixtures of pollutants in utero have reported reductions in birth weight, apparently attributable to both premature birth and IUGR, as well as variations in other size measures, such as length and head circumference at birth. More recent studies have used correlations with PAH-derived DNA adducts and the differential impact of pollution sources with high versus low PAH levels to more clearly establish the role of PAHs on LBW.

Perera and colleagues (1998) studied developmental effects of fetal exposure to PAHs through ambient pollution from burning coal in Poland (Table 8.12). Plasma cotinine and PAH-DNA adducts in leukocytes were measured in umbilical cord blood as dosimeters of cigarette smoke and transplacental PAH, respectively. Newborns whose levels of PAH-DNA adducts were above the median (3.85 per 10^8 nucleotides) had a significantly decreased birth weight, length, and head circumference. Cotinine was also significantly and inversely associated with birth weight and length. Similarly, Dejmek and colleagues (2000) studied birth outcomes in relation to air pollution in two towns in the Czech Republic, one industrialized (Teplice) and one rural (Prachatice) (Table 8.12). The authors defined IUGR as a birth weight below the 10th percentile by gender and gestational week. In Teplice, IUGR was observed in 9.6 percent of pregnancies, while at Prachatice, 8.2 percent were affected. There was a significant association of IUGR with exposure to air pollution, particularly to PAHs rather than to particulate matter (Table 8.12). The association between PAH exposure and IUGR was only significant during the first month of gestation. The AOR was 1.63 (95 percent CI, 0.87–3.06) for a medium exposure and 2.39 (95 percent CI, 1.01–5.65) for a high exposure. Researchers interpreted these findings as indications that the induction of IUGR by a PAH exposure resulted from an early developmental effect.

Despite the exposures to mixed pollutants, these studies provide specific correlations of impacts on birth weight and development with PAH exposure either through a determination of specific DNA adducts or on the basis of differential exposures to PAHs and other pollutants. Extensive evidence from other studies shows an impact of air pollution on birth weight and other pregnancy outcomes (Šrám et al. 2005), supporting the plausibility of a causal relationship between LBW and exposure to PAHs, in spite of the difficulties in assigning causality to specific compounds within mixtures.

Animal studies also show developmental delay and LBW after in utero exposure to pure PAHs, thus strengthening the epidemiologic data. MacKenzie and Angevine (1981) reported statistically significant reductions in weights of exposed pups compared with those of controls for all in utero doses of B[*a*]P, as well as evidence of a progressive dose response. Similarly, Bui and colleagues (1986) observed reductions in fetal weight in a mechanistic study that compared the effects of B[*a*]P with those of methadone, another known fetotoxicant. These authors also observed reductions in uterine weight among the pregnant dams, which suggest that B[*a*]P treatment affects both the fetus and the maternal system.

Developmental Immunotoxicity

PAH exposures have a variety of effects on the immune system. Extensive literature describes the effects of exposure to pollutant mixtures containing PAHs in adult humans. Researchers have demonstrated interest in performing studies on the initiation and exacerbation of asthma and other allergic respiratory conditions from exposure to diesel exhaust, often in combination with other allergens (Riedl and Diaz-Sanchez 2005). Findings suggest that these exposures can potentiate allergic reactions to antigens such as pollen. Exposure early in life may result in a shift in T-cell activity patterns toward a more atopic profile. Studies have also linked these effects to

exposures to other pollutant mixtures that contain PAHs, including tobacco smoke (NCI 1999). Literature reviews also describe mechanistic studies of similar processes in animals. As noted earlier, the presence of PAHs in these pollutant mixtures raises the likelihood that these substances are among the causative agents. However, it is not generally possible to separate the PAH effects from those of other components in the mixture. Synergistic interactions among these components may also be a significant factor.

In contrast to the stimulatory or adjuvant effects at comparatively low exposure levels, higher doses of PAHs in humans have immunosuppressive effects (Karakaya et al. 2004). Similar findings and investigations of the mechanisms involved have been extensively described in adult animals (Table 8.12). The primary effects noted after fetal and neonatal exposures involved immunosuppression, which is often profound and persistent (Urso and Gengozian 1982; Urso and Johnson 1988; Urso et al. 1992), and includes both selective and overall reductions in various cellular components of the immune system, particularly T cells (Holladay and Smith 1994; Lummus and Henningsen 1995). Holladay and Luster (1996) have reviewed the effects of B[*a*]P on T-cell development and the long-term consequences on the development of the immune system. Although development of the immune system begins in utero, important structural and functional changes occur after birth. In view of this continuing development, enhanced sensitivity to exposures to immunotoxicants both during gestation and infancy is to be expected.

Toxic Effects of Naphthalene

Studies have not widely reported or characterized naphthalene as a cause of toxic effects on reproduction and fetotoxic or teratogenic effects. However, Plasterer and colleagues (1985) did report a slight reduction in the number of pups per litter, as well as toxic effects in the mother after high oral doses of naphthalene, that is, the lethal dose for 50 percent of the population (LD_{50}). However, the reports of preferential toxic effects in the neonate or infant described here are examples of toxic effects on postnatal development.

Researchers have reported hemolysis in infants exposed to very high doses of naphthalene (Siegel and Wason 1986). This effect appears to be caused by the metabolites (1- and 2-naphthol and 1- and 2-naphthoquinones) that produce methemoglobinemia. Also, naphthalene damaged both ciliated and Clara cells of the bronchiolar epithelium in mice (Plopper 1992a,b; Van Winkle et al. 1995). Neonatal mice were more sensitive to this damage than were adult mice (Fanucchi et al. 1997). Although the experiment involved intraperitoneal dosing, the effects appear to depend on the metabolism of naphthalene in the target tissues and are therefore probably independent of the route of administration.

Endocrine Disruption

Many investigators have reported that PAHs disrupt reproductive and developmental events and other physiological processes under endocrine control. Some of these effects appear to reflect direct action on hormones and their receptors as opposed to the cytotoxic action of reactive metabolites noted earlier. The nuclear AH receptor is responsible for regulating several cytochrome P-450 isoenzymes and triggering their induction in the presence of various xenobiotics, including PAHs, chlorinated dioxins, and coplanar polychlorinated biphenyls. This receptor also appears to have a range of other functions, including the modulation and proliferation of cell growth. The role of this receptor in the regulation of apoptosis, which includes the *BAX* gene product, has already been noted in the context of oocyte loss and may be involved in other processes in which apoptosis occurs.

The AH receptor also appears to interact with and in some cases control the expression of other nuclear receptors. B[*a*]P reduces the expression of receptors for the epidermal growth factor and the in vitro secretion of chorionic gonadotropin by human placental cells (Zhang et al. 1995). PAHs reportedly have antiestrogenic effects. Chaloupka and colleagues (1992) used MCF-7 human breast cancer cells to investigate in vitro the inhibition by 3-methylcholanthrene (a synthetic PAH) of estradiol-stimulated cell growth. Subsequently, Navas and Segner (2000) described antiestrogenic effects of various PAHs on synthesis of vitellogenin in cultured hepatocytes of rainbow trout by 17-β-estradiol. In both systems, the binding of the PAH to the AH receptor appears to be the event that triggers a range of cellular responses, including a reduced expression of the estrogen receptor.

Other Compounds

Tobacco smoke contains thousands of compounds, some of which have also been identified as known or suspected reproductive toxicants, in addition to the compounds already described here in detail. Examples include toluene, carbon disulfide, dichlorodiphenyltrichloroethane, styrene, benzene, and vinyl chloride.

Other compounds that are less well studied may also influence reproductive outcomes. As noted earlier in this chapter (see "Tubal Function"), cigarette smoke impairs oviductal functioning (Knoll and Talbot 1998) and inhibits the growth of the chick chorioallantoic membrane (CAM)

(Melkonian et al. 2000, 2002). To identify which chemicals in tobacco smoke are responsible for these toxic effects, solutions of mainstream smoke were fractionated and the eluates were screened for inhibitory activity in the CAM and oviductal assays. The CAM assay measures CAM and embryonic growth, and the oviductal assays measure ciliary beat frequency, oocyte pickup rate, and rate of smooth muscle contraction. The chemicals in each eluate that retained 80 percent or more of the inhibitory activity were identified by gas chromatography and mass spectrometry. This approach identified pyridine, pyrazine, phenol, indole, and quinoline derivatives as the major groups of inhibitory compounds (Ji et al. 2002; Melkonian et al. 2003; Riveles et al. 2003, 2004, 2005). Members of each group were highly effective in both the CAM and oviductal assays that measure diverse biologic processes. In every group, some chemicals were inhibitory at nanodoses and picomolar doses, and indole was inhibitory in the oviductal assays at femtomolar doses. In general, methyl and ethyl substitutions increased the toxicity of these compounds. Some of the inhibitory chemicals (e.g., 3-ethylpyridine and pyrazine) are on the Flavor and Extract Manufacturer's Association's Generally Recognized as Safe list and the "Everything" Added to Food in the United States list from the U.S. Food and Drug Administration (FDA); some are added to cigarettes to enhance flavor.

Other Molecular Mechanisms

In addition to the molecular mechanisms of specific toxins outlined here, researchers have conducted general investigations of smoking in relation to pathways for molecular mechanisms.

Genetic Damage to Sperm

Concern exists that exposures to toxins such as those in cigarette smoke may cause damage to sperm DNA that could be transmitted to offspring (Chapin et al. 2004). This important question of male-mediated toxic effects on development can now be addressed directly through use of tools of molecular genetics that detect and measure chromosomal changes and DNA damage in ejaculated sperm cells (Perreault et al. 2003). Studies have reported significant increases in sperm DNA and chromatin damage, including oxidative DNA damage, in smokers compared with nonsmokers from both infertility clinic and nonclinic populations (Fraga et al. 1996; Shen et al. 1997); strand breaks (Sun et al. 1997; Potts et al. 1999); native DNA stainability (Sofikitis et al. 1995; Spanò et al. 1998); denaturation of labile sites (Potts et al. 1999); DNA adducts (Zenzes et al. 1999a,b; Horak et al. 2003); and apoptosis (Belcheva et al. 2004). Only a few studies did not find statistically significant differences related to exposure to cigarette smoke. One of these studies focused on DNA strand breaks (Sergerie et al. 2000) and another on oxidative DNA damage (Loft et al. 2003). Both studies were conducted in nonclinical populations.

Researchers have determined a statistically significant trend across published studies ($p < 0.001$) for sperm aneuploidy associated with smoking (Robbins et al. 2005). Sperm carrying aberrant chromosomes are capable of fertilizing eggs that result in aneuploid offspring. For example, the father contributes the extra Y chromosome in 100 percent of XYY offspring, the extra chromosome in approximately 30 to 50 percent of XXY offspring with Klinefelter syndrome, and in up to an estimated 80 percent of the offspring with missing X in Turner's syndrome (Hassold 1998; Martínez-Pasarell et al. 1999). In addition to congenital anomalies, damage to genetic material could affect sperm quality and could be manifested as infertility or very early pregnancy loss if the damage is incompatible with survival.

Nutrient Deficiencies

Micronutrients

Deficiencies of micronutrients may contribute to adverse pregnancy outcomes, and smoking could act through this relationship. As previously mentioned, a decrease in the amount of collagen III likely leads to a weakening of the tensile strength of amniotic membranes, which could increase the risk of PPROM. Vitamin C is required for collagen formation in amnion epithelial cells, and studies have noted reduced vitamin C in women with PPROM (Wideman et al. 1964; Casanueva et al. 1993). Studies consistently show that plasma vitamin C levels are lower in smokers than in nonsmokers, a finding that appears to be attributable to a lower intake as well as increased utilization in the body (Preston 1991; Lykkesfeldt et al. 2000; Cogswell et al. 2003). Vitamin C levels in the amniotic fluid of smokers are also lower than those in nonsmokers (Barrett et al. 1991). Vitamin C is important for normal immune functioning. Deficiencies of vitamin C are associated with impaired immunocompetence, reduced counts of polymorphonuclear leukocytes, phagocytosis, and depressed cell-mediated immunity (Long and Santos 1999). A vitamin C deficiency in smokers could contribute to adverse pregnancy outcomes by impairing maternal immune responses to genital tract infections. Data on other antioxidant levels in smokers are conflicting (Cogswell et al. 2003).

Zinc deficiency may also play a causal role in PPROM and other adverse outcomes. Zinc is necessary for DNA

synthesis, transcription, and translation, as well as cell division and cell growth (Fisher 1975; Vallee and Falchuk 1993; Prasad 1996). Low zinc levels result in impaired immune function (Fraker and King 2004), increased susceptibility to infectious diseases (Fischer Walker and Black 2004), and cell death (Fraker 2005). Researchers have found reduced serum and amniotic fluid levels of zinc in pregnant women with PPROM (Anderson 1979; Kiilholma et al. 1984). A prospective study of pregnant women associated a low zinc intake with a threefold increase in PPROM (Scholl et al. 1993). Some data suggest that smokers are more likely to experience a zinc deficiency than are nonsmokers (Cogswell et al. 2003). Levels of maternal dietary zinc, plasma zinc, and zinc in red blood cells are similar in smokers and nonsmokers close to the time of delivery. However, shortly after delivery, zinc levels in cord blood and polymorphonuclear cells, which may be a more sensitive indicator of zinc depletion, were lower in smokers than in nonsmokers (Simmer and Thompson 1985; Kuhnert et al. 1987b). Cadmium, which accumulates in the placenta and binds to zinc, may contribute to a local zinc deficiency in smokers (Kuhnert et al. 1987a,b, 1988a,b; Preston 1991).

Amino Acids

In addition to a supply of nutrients delivered to the fetus through the uteroplacental circulation, fetal growth depends on nutrient transport across the syncytiotrophoblast. Because the placenta is impermeable to most proteins, almost all of the fetal proteins are synthesized by the fetus from amino acids supplied by the mother. Amino acids from the mother's blood are taken up by the active transport of placental trophoblasts and then diffused into the umbilical venous blood. There is accumulating evidence that abnormalities in amino acid transport across the placenta can contribute to impaired fetal growth (Pastrakuljic et al. 1999). In addition, many studies suggest that maternal smoking adversely affects this transport, and this effect may be one mechanism by which smoking restricts fetal growth. Levels of several amino acids in fetal plasma, umbilical plasma, and placental villi are lower in smokers than in nonsmokers (Jauniaux et al. 1999, 2001) (see "Carbon Monoxide" and "Nicotine" earlier in this chapter). Additional research is needed to determine more precisely how the results of the in vivo and in vitro studies may be related to adverse pregnancy outcomes among maternal smokers.

Nitric Oxide Activity

Researchers have also studied the effects of smoking on NO activity. Endothelial NO is a potent vasodilator synthesized by NO synthase in vascular endothelial cells (ENOS) (Moncada and Higgs 1993). NO regulates blood pressure by its effects on vascular resistance. Evidence suggests that a decrease in the release of basal NO may predispose a person to hypertension, vasospasm, and thrombosis, whereas elevated levels may be associated with shock (Moncada and Higgs 1993; Änggård 1994; Cooke and Dzau 1997; Oemar et al. 1998). In pregnancy, NO is also present in placental villi and is believed to play an important role in the vasodilatory response of the maternal, uteroplacental, and fetoplacental circulatory systems (Myatt et al. 1991, 1992; Poston et al. 1995). Reductions of NO activity in placental villi from pregnancies with preeclampsia and IUGR suggest a role for NO in pregnancy complications (Sooranna et al. 1995). In vitro research indicates that the mRNA and the protein expression of ENOS are decreased in endothelial cells from preeclamptic pregnancies (Wang et al. 2004).

Studies have associated maternal smoking with a dose-dependent decrease in endothelial-dependent vessel dilation (Lekakis et al. 1998; Poredoš et al. 1999) and with an inhibition of ENOS activity, depending on the *ENOS* genotype (Wang et al. 2000b). Researchers have reported that endothelial cells from the umbilical cords of infants born to smokers had a 40-percent reduction in ENOS activity and a 32-percent reduction in ENOS levels compared with those in cells from infants born to nonsmokers. Furthermore, the ENOS activity level was associated with the number of cigarettes smoked per day (Andersen et al. 2004). The effects of maternal smoking on ENOS activity could lead to lower NO levels, resulting in a loss of dilatory capacity and contributing to IUGR.

Smoking and Maternal and Neonatal Genetic Polymorphisms

Investigators have reported differences in the human metabolism of toxic constituents in tobacco smoke (Benowitz et al. 1999; Lee et al. 2000; Yang et al. 2001). These metabolic differences may reflect a differential induction of toxins and drug-metabolizing enzymes, such as phase I cytochrome P-450; phase II glutathione-*S*-transferase (GST); NAT1 and 2; placental alkaline phospholipase; lysyl oxidase; the platelet-activating factor acetylhydrolase; TGFα; TGFβ3; and microsomal epoxide hydrolase. Genetic polymorphisms that alter the expression of these enzymes are in the pathway of development of disease such as lung cancer. These polymorphisms also appear to modify the relationship between prenatal exposure to tobacco smoke and birth outcomes. Studies of prenatal exposure to fetotoxins present in tobacco smoke (e.g., PAHs, AH, and benzene) have linked these toxins

to adverse pregnancy outcomes through the inducibility of phase I enzymes such as CYP1A1 (Huel et al. 1993; Lagueux et al. 1999; Dejmek et al. 2000; Wang et al. 2000a), which can vary by host genotype.

Birth Defects

Initial investigations of the mechanisms of maternal or neonatal metabolism of tobacco smoke toxins and adverse birth outcomes were conducted in studies of birth defects. Several studies examined the potential interaction of maternal exposure to tobacco smoke and maternal and/or neonatal genotypes in association with orofacial cleft in newborns (Table 8.13). The genetic polymorphisms that code for the expression of tissue damage, inflammatory response, and immune mediator enzymes and that were examined in those studies include *TGFα* and *TGFβ3*, *MSX1*, and *EPHX1*, as well as gene variants of both phase I activation and phase II detoxification enzymes *CYP1A1, GSTM1, GSTT1, NAT1,* and *NAT2*. Prenatal exposure to tobacco smoke was measured by self-reports of maternal active smoking, maternal exposure to secondhand smoke, and paternal active smoking. Most of these studies examined the *TGFα* genotype in neonates. In one study, genotyping was performed in both neonates and parents.

A case-control study of infants with a *TGFα *TAQ1* genotype that contained a rare allele and whose mothers had smoked during pregnancy found a significantly elevated risk for cleft palate in offspring (Table 8.13) (Hwang et al. 1995). In a large, population-based, case-control study conducted by the California Birth Defects Monitoring Program registry, the risks of cleft palate and cleft lip with or without cleft palate were significantly elevated among White infants with *TGFα *rare* genotypes (**A2)* whose mothers were heavy smokers (Shaw et al. 1996). However, three subsequent case-control studies (Christensen et al. 1999; Romitti et al. 1999; Beaty et al. 2001) that failed to replicate these findings had fewer cases and one study had used a lower cutpoint for smoking than that used by Shaw and colleagues (1996). None of the five studies cited above presented regression models with terms for estimating maternal smoking levels and the *TGFα* genotype interactions. Zeiger and colleagues (2005) conducted a meta-analysis of data from these five studies and found a marginally significant interaction between maternal smoking and infant *TGFα* *allele genotypes (**A2*) in relation to cleft palate (OR = 1.95 [95 percent CI, 1.22–3.10]). There was no evidence of an interaction in relation to cleft lip and cleft palate (OR = 0.86 [95 percent CI, 0.53–1.40]).

Romitti and colleagues (1999) also examined the *TGFβ3* genotype and maternal smoking in relation to the risk of cleft palate or cleft lip and cleft palate (Table 8.13). These researchers found a significantly elevated risk for the conditions among infants who were homozygous for the common **1* allele at the X5.1 or 5′UTR.1 site and whose mothers had smoked 10 or more cigarettes per day. There was no evidence of an interaction for infant genotypes that included the rare **2* allele.

Hartsfield and colleagues (2001) did not observe any significant interaction between maternal smoking and *EPHX1* (codon 113) or *null GSTM1* genotypes in a case-control study of isolated cleft lip and cleft palate (Table 8.13). van Rooij and colleagues (2001) examined the association of maternal prenatal smoking and the maternal *GSTT1* genotype. The researchers found that mothers who smoked and carried the *GSTT1 null* genotype had a marginally higher risk for delivering an infant with oral clefting than that of nonsmokers who carried the wild-type genotype. Although the RR was not statistically significant, it was almost five times greater when both mothers and their infants carried the *GSTT1 null* genotype. There was no evidence of an interaction between maternal smoking and the *CYP1A1* genotype with a recessive allele in relation to oral clefting. In a case-control study, the *CYP1A1*, *GSTT1*, and *GSTM1* polymorphisms were also examined as risk factors for hypospadias, a congenital anomaly of the male reproductive organs (Kurahashi et al. 2005). The study did not observe any increased risk of hypospadias among children born to mothers who smoked and had various genotypes, including *CYP1A1 *MSPI* variant allele genotype or the *GSTT1 null* genotype or *GSTM1 null* genotype. In a case-only, haplotypic analysis of an intronic CA repeat of the *MSX1* gene in 206 infants with oral clefting, there was evidence for an interaction with maternal prenatal smoking (Fallin et al. 2003). In the Iowa study (Romitti et al. 1999), infants whose *MSX1 X1.3* or *MSX1 X2.4* genotype contained the **2* allele and whose mothers smoked 10 or more cigarettes per day also had a significantly elevated risk of cleft palate (Table 8.13). In a study of limb deficiency defects, Carmichael and colleagues (2004) did not observe any significantly elevated risk for infants with *MSX1* intronic CA repeat genotype whose mothers smoked during pregnancy. In another case-control study from the California Birth Defects Monitoring Program, the *NAT1 1088* genotype **A/*A* and the *NAT1 1095* genotype **A/*A*, but not *NAT2* polymorphisms, were strongly associated with isolated oral clefting in infants whose mothers had smoked during pregnancy (Table 8.13) (Lammer et al. 2004).

Other Reproductive Endpoints

Several studies have also examined the potential interaction among phase I and II toxins, genes for drug metabolism, and prenatal exposure to tobacco smoke in

Table 8.13 Studies of interactions between genotype and exposure to tobacco related to oral clefting

Study	Study period	Population	Definition of smoking	Key results
Hwang et al. 1995	1984–1992	69 case infants with CP 114 case infants with CL/P 284 control infants with no cleft defects	Pregnant smokers Pregnant nonsmokers Light smokers: ≤10 cigarettes/day Heavy smokers: >10 cigarettes/day	• Among White infants with *TGF*α genotype containing the **A2* (*rare*) allele at the *TAQ1* site whose mothers were heavy smokers, risks of CP and CL/P were significantly elevated (OR = 5.60 [95% CI, 1.36–22.9]) in infants with no family history of birth defects • By amount smoked, risk for CP was elevated among infants with **A2* allele born to light smokers (OR = 6.16 [95% CI, 1.09–34.7]) as well as infants whose mothers were heavy smokers (OR = 8.69 [95% CI, 1.57-47.7])
Shaw et al. 1996	1987–1989	731 case infants with orofacial clefting 348 with isolated CL/P 141 with isolated CP 99 with multiple CL/P 74 with multiple CP 69 with known cause 939 control infants California Birth Defects Monitoring Program	Smokers: 1–19 cigarettes/day, ≥20 cigarettes/day Nonsmokers exposed to secondhand smoke from anyone inside mother's home who smoked daily during 4 months after conception or from regularly frequented places where others smoked	• White infants whose *TGF*α genotype contained the **A2* allele born to maternal heavy smokers had increased risk of isolated CL/P (OR = 6.1 [95% CI, 1.1–36.6]) • Risks were also elevated in the same group for isolated CP (OR = 9.0 [95% CI, 1.4–61.9]) and for "known etiology clefts" (OR = 10.8 [95% CI, 1.2–111.7]) • Sample size in non-White racial groups was too small to permit reliable estimates of effect
Christensen et al. 1999	1991–1994	316 case infants 233 with CL/P 83 with CP 604 control infants	Smokers (before conception and/or during pregnancy): mean cigarettes/day Nonsmokers	• No evidence was found for increased risk of CP or CL/P among infants with the **rare* allele for *TGF*α whose mothers had smoked while pregnant
Romitti et al. 1999	1987–1994	225 case infants 161 with CL/P 64 with CP 393 control infants	Smokers (before conception and/or during pregnancy): 1–9 cigarettes/day, ≥10 cigarettes/day Nonsmokers	• For infants whose genotype contained the **rare* allele for *TGF*α or *TGFβ3,* maternal smoking did not increase risk of having CP or CL/P • Infants whose genotype contained common **1* allele for *TGFβ3* (*X5.1* or *5′UTR.1* sites) were at increased risk of having CP or CL/P • With maternal smoking of ≥10 cigarettes/day, infants whose *MSX1 X1.3* genotype contained **A2* allele had elevated risk of CP (OR = 2.7 [95% CI, 1.1–6.3]) • Similar results were found among infants whose *MSX1 X2.4* genotype contained **A2* allele (OR = 2.8 [95% CI, 1.1–6.9])

Table 8.13 Continued

Study	Study period	Population	Definition of smoking	Key results
Beaty et al. 2001	1992–1998	175 infants 111 with CL/P 64 with CP 182 control infants	Smokers (before conception and/or during pregnancy) Nonsmokers (survey of passive exposure to cigarette smoke)	• No evidence was found for increased risk of isolated CL, CP, or CL/P among infants with **rare* allele for *TGFα* whose mothers had smoked while pregnant
Hartsfield et al. 2001	1987–1989	85 case infants with CL/P 110 control infants	Pregnant smokers	• With maternal smoking, infants whose genotype was homozygous for *EPHX1* codon 113 **H* allele were not at higher risk of CL/P than were infants with *EPHX1* codon 113 wild-type allele (OR = 0.4 [95% CI, 0.1–1.3])
van Rooij et al. 2001	Not reported	113 infants with nonsyndromic oral clefts 104 control infants	Smokers (before conception and/or during pregnancy and/or postpartum) Nonsmokers	• Attributable fraction for oral clefting in maternal smokers with *GSTT1 null* genotype was 7.3%, increased to 7.7% when both mother and infant had *GSTT1 null* genotype • No interaction was observed with maternal smoking and *CYP1A1* polymorphism (maternal or infant) associated with risk of oral clefting
Fallin et al. 2003	1992–1998	206 case infants with oral clefting No controls	Smokers (before conception and/or during pregnancy) Nonsmokers (survey of passive exposure to secondhand smoke)	• Case-only (combined CP and CL/P) tests of association between *MSX1* haplotypes and maternal smoking during first trimester showed excess cases of haplotype containing intronic CA repeat marker among infants of mothers who smoked
Lammer et al. 2004	1987–1989	437 case infants 309 with isolated CL/P 128 with CP California Birth Defects Monitoring Program	Smokers: 1–19 cigarettes/day or ≥20 cigarettes/day Nonsmokers exposed to secondhand smoke from anyone inside mother's home who smoked daily during 4 months after conception or from regularly frequented places where others smoked	• *NAT1 1088* homozygous **A/*A* genotype among infants whose mothers smoked was strongly associated with CL/P (OR = 3.9 [95% CI, 1.1–17.2]) • *NAT1 1095* homozygous **A/*A* genotype was also strongly associated with CL/P (OR = 4.2 [95% CI, 1.2–18.0]) • No significant interaction was observed for *NAT2* polymorphisms and maternal smoking in relation to CL/P or CP
Zeiger et al. 2005	1984–1998 Meta-analysis of data from 5 studies	726 case infants with CL/P 335 with CP 1,565 control infants (meta-analysis)	Smokers (before conception and/or during pregnancy and/or postpartum) Nonsmokers	• Meta-analysis results indicated that with maternal smoking at any level during pregnancy, risk of CP increased for infants whose *TGFα* genotype contained **A2* allele (OR = 1.95 [95% CI, 1.22–3.10]) • No significant interaction for maternal smoking and infant carrying *TGFα *A2* allele was associated with CL/P

Note: **CI** = confidence interval; **CL/P** = cleft lip with or without cleft palate; **CP** = cleft palate; **OR** = odds ratio.

Table 8.14 Decreased birth weight, preterm delivery, intrauterine growth retardation, and neonatal oxidative damage: interactions between host genotype and exposure to tobacco smoke

Study	Study period	Population	Definition of smoking	Key results
Hong et al. 2001	1999	81 maternal and infant pairs measured for neonatal oxidative damage	Nonsmokers exposed and unexposed to secondhand smoke during pregnancy, determined by measuring cotinine levels	• Urinary concentrations of neonatal oxidative damage from 8-OH-dG were correlated with maternal exposure to secondhand smoke • Infants of mothers with *GSTM1 null* genotype and exposure to secondhand smoke had significantly higher concentrations of 8-OH-dG than did infants of mothers with *GSTM1 present* genotype and no exposure to secondhand smoke
Wang et al. 2002	1998–2000	207 mothers of preterm and/or LBW infants 534 control infants	Smokers (continuously or with cessation during pregnancy) Self-reported lifetime nonsmokers	• Mothers with *CYP1A1 MSPI* genotype containing the **a* allele who smoked throughout pregnancy had infants with mean birth weight decrements of -520 g compared with mothers with *CYP1A1 MSPI *A/*A* genotype who never smoked (from stratified models: LBW OR = 3.2 [95% CI, 1.6–6.4]; from interaction term model: LBW OR = 3.1 [95% CI, 1.2–7.8]) • Infants of mothers with *GSTT1 null* genotype who smoked throughout pregnancy had mean birth weight decrements of -642 g compared with mothers with the *GSTT1 present* genotype who never smoked (from stratified models: LBW OR = 3.5 [95% CI, 1.5–8.3]; from interaction term model: LBW OR = 2.4 [95% CI, 0.9–6.6]) • Among mothers with *CYP1A1 MSPI *Aa/*aa* and *GSTT1 null* genotypes, infants of mothers who smoked throughout pregnancy had mean birth weight decrements of -1,285 g (test of interaction: -1086 g, $p < 0.001$) • Interaction between maternal *GSTT1* genotype and maternal smoking in relation to preterm birth was significant (OR = 2.9 [95% CI, 1.0–8.2]) • Interaction between maternal *CYP1A1 MSPI* genotype and maternal smoking in relation to intrauterine growth retardation was significant (OR = 3.0 [95% CI, 1.2–7.8])
Hong et al. 2003	1999	266 maternal and infant pairs measured for birth weight	Nonsmokers exposed or unexposed to secondhand smoke during pregnancy, determined by measuring cotinine levels	• Mothers exposed to secondhand smoke during pregnancy and having *GSTM1 null* genotype delivered infants with mean birth weight decrements of -158 g compared with unexposed women with *GSTM1 present* genotype, after adjustment for gestational age • Similarly, women exposed to tobacco as secondhand smoke and having *GSTT1 null* genotype delivered infants with mean birth weight decrements of -203 g compared with unexposed women with *GSTT1 present* genotype, after adjustment for gestational age

Table 8.14 Continued

Study	Study period	Population	Definition of smoking	Key results
Magrini et al. 2003	NR	214 maternal and infant pairs measured for birth weight	Smokers Nonsmokers	• Placental specimens from maternal smokers who did not have *PLAP *1/*1* genotype were associated with elevated risk of LBW infants (OR = 6.02 [95% CI, 1.65–23.02]) • This effect was more marked in mothers aged >28 years
Nukui et al. 2004	NR	955 mother and infant pairs assessed for preterm birth and LBW	Smokers (cigarettes/ day for first and third trimesters) Nonsmokers (hours of exposure to secondhand smoke)	• Maternal smokers during third trimester and their infants, both with *GSTT1 null* genotype, had higher risk of premature birth than did mothers and infants with *GSTT1 present* genotype among nonsmokers in third trimester (OR = 4.0 [95% CI, 1.7–9.5]) • When both mother and infant had *GSTT1 null* genotype, the risk of premature birth was elevated for smokers and nonsmokers and their infants

Note: **8-OH-dG** = 8-hydroxy-2′-deoxyguanosine; **CI** = confidence interval; **g** = grams; **LBW** = low birth weight; **NR** = data not reported; **OR** = odds ratio.

relation to other outcomes, such as LBW, preterm birth, IUGR, and neonatal oxidative damage (Table 8.14).

A large case-control study described an interaction between self-reported smoking during pregnancy and maternal genetic polymorphisms for *CYP1A1 MSPI* and *GSTT1*, which were associated with reduced birth weight, preterm delivery, reduced gestation, and IUGR (Table 8.14) (Wang et al. 2002). Mothers who had smoked continuously during pregnancy and who were heterozygous variant type (**A/*a*) or homozygous variant type (**a/*a*) for *CYP1A1 MSPI*, were at a threefold higher risk of having a LBW (<2,500 g) infant compared with lifetime nonsmokers who were homozygous wild type (**A/*A*). In these genotype-exposure groups, gestation was reduced on average by 1.5 weeks among smokers who were *CYP1A1 MSPI *A/*a* or **a/*a*. Smokers with the *GSTT1 null* genotype gave birth to infants with significant decrements in birth weight compared with birth weight of infants of nonsmokers who carried at least one *GSTT1* allele. The risk of growth retardation, defined as less than 85 percent of the ratio of observed birth weight to mean birth weight for gestational age, was associated with smoking in mothers who carried the *CYP1A1 MSPI *A/*a* or **a/*a*. This study was unique because it presented regression models that evaluated interaction terms along with analyses stratified by gene variant and smoking status, but it lacked an objective measure of smoking.

In a study conducted in Korea, Hong and colleagues (2003) examined the potential interaction of the *GST* family gene variants and exposure to secondhand smoke in association with mean birth weight (Table 8.14). Women classified as exposed to secondhand smoke, as determined by assays of urinary levels of cotinine, who carried the *GSTM1 null* genotype delivered infants with a mean birth weight decrement of -158 g, after adjustment for gestational age. A similar pattern was observed among mothers with the *GSTT1 null* genotype (decrement of -203 g). In a subsample of 81 women in the same study population, Hong and colleagues (2001) examined the effect of exposure to secondhand smoke and maternal *GST* family genotypes in relation to measurements of neonatal oxidative damage as urinary levels of 8-hydroxy-2′-deoxyguanosine (8-OH-dG). Infants born to women classified as exposed to secondhand smoke who carried the *GSTM1 null* genotype had significantly higher levels of log urinary 8-OH-dG (geometric mean level = 4.03 [95 percent CI, 2.13–7.61]) than did women classified as unexposed who carried the *GSTM1* wild type. Significant differences in 8-OH-dG remained after adjusting for confounders.

One study evaluated phase I and II metabolic enzyme gene variants (Table 8.14) (Nukui et al. 2004). The authors reported that the most significantly elevated risk of premature birth occurred when both mother and infant carried the *GSTT1 null* genotype. The results were the same for smokers and nonsmokers, so no interaction was observed. Phase I genetic polymorphisms (e.g., *CYP1A1*) were not associated with an elevated risk of a premature birth. A study that examined *PLAP* polymorphisms found an elevated risk of LBW when the *PLAP *1/*1* genotype was absent in mothers who smoked (Magrini et al. 2003).

Implications

The evidence of a causal relationship between smoking during pregnancy and increased risk for adverse pregnancy outcomes is sufficient to warrant promoting smoking cessation among women early in pregnancy or before they become pregnant, because the critical period may be quite early. For example, the impact of smoking on oral clefts could be lessened by 5 to 22 percent if women stopped smoking before pregnancy. Thus, there is a need for widespread implementation of interventions for effective smoking cessation that target all women of childbearing age.

Smoking may have several ramifications of alterations in fertility, menstrual cycle function, or sperm quality, including a burden on the health care system and loss of work productivity. These changes may affect a woman's ability to conceive and maintain a pregnancy at a desired time in a couple's life. The reproductive life span may be shortened, and early menopause is associated with other hormone-related health problems such as osteoporosis and cardiovascular disease (Harlow and Ephross 1995; Sowers and La Pietra 1995; Cooper and Sandler 1998). Women with short menstrual cycles may also be at a higher risk of breast cancer (Kelsey et al. 1993). Such effects warrant smoking cessation early in the reproductive age span, or ideally, prevention of smoking initiation among youth.

Smoking Prevention and Cessation

Smoking cessation is one of the most important actions a woman can take to improve the outcome of her pregnancy, and most women who stop smoking during pregnancy do so on their own. Because women know about the adverse effects of smoking on their health and that of a fetus, pregnancy may be a time when smoking cessation efforts and interventions are potentially more effective (Mullen 1999; Fiore et al. 1996, 2008). Nevertheless, most smokers do not stop smoking during pregnancy. Tobacco addiction is progressive and chronic and, in consequence, smoking cessation interventions focusing on the prenatal period have failed to achieve long-term abstinence among most pregnant smokers. Two-thirds of women who smoke during the first pregnancy also smoke during the second, exposing the first infant to tobacco smoke both in utero and postnatally (Dietz et al. 1997).

Population-based, cross-sectional surveys have been widely used to monitor prenatal smoking rates (Connor and McIntyre 1999; Owen and Penn 1999; Ebrahim et al. 2000). However, such data do not provide information on changes in smoking behaviors during pregnancy, in contrast to data collected during prenatal care, in which smoking behaviors are recorded on more than one occasion, as described by Kirkland and colleagues (2000). Such longitudinal data can usefully supplement survey data to monitor progress in control of prenatal exposure to tobacco smoke. Because of the social desirability of nonsmoking status, which is greater during pregnancy, the actual prevalence of smoking may be even higher than is self-reported. In the United Kingdom, 16 percent of respondents to a survey reported that they did not admit to their physicians that they smoked (*Bulletin of the World Health Organization* 1999). A study in the United States found nondisclosure rates of 28 percent at enrollment into prenatal care and 35 percent at follow-up (Kendrick et al. 1995). Therefore, biochemical verification of smoking status of each woman during each contact with a clinician is needed to evaluate cessation interventions.

Clinicians who provide health care to women have an important role in reducing the burden of smoking among women. Clinically proven programs for smoking cessation that can be delivered in primary care settings are now available. However, there is a dearth of information specifically addressing the most effective smoking prevention and cessation interventions for women of childbearing age, especially those of low socioeconomic status. For this reason NC1 has implemented the TReND: Low SES Women and Girls Project. This project was created to strategically address and examine the effects of multiple tobacco control policies on diverse populations of low SES women and girls. In addition, the USDHHS Office of Women's Health has developed an interagency work group to address this issue. The FDA Office of Women's Health has also developed a guide for providers to educate women about medications available to assist them if they are ready to quit smoking.

Experts attending the 1998 Consensus Workshop on Smoking Cessation During Pregnancy reviewed the evidence related to counseling on smoking cessation during pregnancy, including the U.S. Public Health Service Clinical Practice Guideline, *Treating Tobacco Use and Dependence* (Fiore et al. 1996; Mullen 1999). This group concluded that brief cessation counseling (5 to 15 minutes), delivered by a trained provider with pregnancy-specific self-help materials, significantly increases rates of cessation among pregnant smokers. The 5A strategy of smoking intervention—Ask, Advise, Assess, Assist, and Arrange—and recommended procedures are outlined in the one-page form, *Brief Smoking Cessation Counseling*

for Pregnant Patients. More intense efforts may be needed for groups of women who are less likely to stop smoking and more likely to relapse (Connor and McIntyre 1999). Smoking cessation interventions should be continued after delivery to prevent relapse, and partners who smoke should be included in such interventions. More than 50 percent of women do not recognize that they are pregnant until after the fourth week of gestation. Therefore, efforts to prevent exposure to tobacco smoke should begin before conception to avoid pregnancy complications or to avoid exposing the fetus to tobacco smoke. Furthermore, to prevent subfertility, cessation efforts should begin even earlier in the reproductive period.

Despite evidence that provider-administered cessation counseling significantly increases rates of cessation among pregnant smokers, evidence suggests that such interventions may do little to decrease overall prenatal smoking prevalence. It has been estimated that universal implementation of a provider-administered psychosocial cessation intervention on a national level would result in only a modest decline (0.8 percentage points) in the overall prevalence of smoking among pregnant women (Kim et al. 2009). Larger reductions in prenatal smoking prevalence will likely require implementation of comprehensive tobacco control policies that effectively decrease smoking prevalence among women of reproductive age.

Unfortunately, pregnant women who smoke most heavily do not appear to respond to the type of behavioral intervention indicated here. The U.S. Public Health Service has suggested, as have others, the need to explore the use of pharmacologic approaches to achieve cessation in women who are unable to stop smoking (Fiore et al. 1996, 2000, 2008). These approaches include nicotine replacement therapy (e.g., gum, patch, inhaler, or spray); nonnicotine products, such as varenicline and bupropion hydrochloride; and second-line pharmacotherapies, such as clonidine and nortriptyline. However, the efficacy and safety of these approaches during pregnancy are not well documented. Pharmacologic interventions should be considered on an individual basis as an adjunct to behavioral interventions. These interventions should be considered for pregnant women only if the increased likelihood of smoking cessation outweighs the harmful effects on the fetus of nicotine replacement therapy and possible continued smoking.

To minimize the effects of smoking among all women and to foster effective perinatal tobacco control, focus and efforts should expand beyond prenatal care to include both the whole family and the entire reproductive life span of women. The complexities associated with smoking cessation among established smokers are underscored by reports of persistent high smoking rates among pregnant women in Canada, the United Kingdom, and the United States (Connor and McIntyre 1999; Owen and Penn 1999; Ebrahim et al. 2000). Long-term reduction in tobacco exposure during pregnancy can be achieved only by encouraging adolescent girls and young women not to start smoking.

Regulation and Policy

Ranking of infant mortality rates in established market economies placed the United States twenty-eighth in 2005 compared with twelfth in 1960. Rates of smoking remain high among women in three categories related to pregnancy—pregnant, planning a pregnancy, and at risk of pregnancy. Substantial efforts would be needed to reduce known risks to pregnancy and infant health, to reverse the stagnant trends in infant mortality rates in the near future. Because only about 20 percent of women successfully control tobacco dependence during pregnancy, smoking cessation is recommended before pregnancy (U.S. Preventive Services Task Force [USPSTF] 2003). National recommendations to support preconception care as an opportunity to reduce maternal risks during pregnancy have been introduced (USPSTF 2003). Therefore, high rates of smoking in the preconception period, almost the same as that among all women of childbearing age, pose both a challenge and an opportunity to the implementation of the preconception care initiative. After childbirth, smoking does not usually decline below the level achieved during pregnancy (USPSTF 2003). Therefore, further challenges to preventing smoking-related harm to infant health include continued postnatal exposure to secondhand tobacco smoke from maternal smoking.

Intervention tools to aid smoking cessation among pregnant women are now available. One report estimated that the costs of implementing such interventions range from $24 to $34 per pregnant smoker counseled (Ayadi et al. 2006). Potential neonatal cost savings that could be accrued for women who stop smoking during pregnancy were estimated at $881 per maternal smoker (Ayadi et al. 2006). A woman's contact with the health care system during and after pregnancy provides enough opportunities to engage women for smoking cessation and provide follow-up and support services to prevent relapse during the interconception period. Furthermore, women of childbearing age in the United States have on average 6.4 health care visits per year (Adams and Marano 1995), and such opportunities can be used to improve access to the 5A strategy of smoking intervention.

Smoking behaviors of partners of women who smoke are important considerations for achieving cessation and prevention of relapse. Extension of services or facilitation

of use of services by the partners would be needed to maintain the benefit from smoking cessation services provided to the women. Because men have fewer contacts with the health care system than do women (Everett et al. 2005), smoking cessation efforts among women provide an opportunity for access to health care for their male partners also for cessation efforts.

Efforts to reduce smoking in the United States have shifted from a primary focus on smoking cessation for individuals to more population-based interventions that emphasize prevention of smoking initiation, reduction of exposure to secondhand tobacco smoke, and policy changes in health care systems to promote cessation. Parallel to these efforts, concerted efforts are needed to reduce the disparity in smoking rates among socially disadvantaged women and White women; targeted efforts aimed at women in the preconception period and those at risk for pregnancy; and efforts to promote smoking cessation among women who are pregnant. One report indicated that bundling of services to address common preventable risks, such as tobacco and alcohol use and risks for sexually transmitted diseases, through a preconceptional approach, would benefit about one-half of all women of childbearing age in the United States (CDC 2006). Tobacco use in women coexists with other risk behaviors or morbidities, including mental health disorders and substance use. Some factors, such as illicit drug use or alcohol use, may synergistically elevate the risk from other factors such as tobacco use or acquisition of sexually transmitted infections, requiring intervention approaches through case management that address more than one risk factor. Use of tobacco and alcohol are comorbidities that can benefit from many efforts to provide several interventions to a woman simultaneously. Such case-management approaches may help to increase adherence to treatment and reduce relapse to smoking after delivery.

Earlier data have indicated that the observed declines during the past four decades in the United States in smoking rates among pregnant women reflect declines that occurred in general among women and were not specific to pregnancy. Therefore, efforts to close the gaps in the diffusion of smoking cessation intervention to individual smokers are still needed and should be a priority. However, large reductions in smoking rates among pregnant women over time are more likely to come from efforts to reduce smoking initiation by young women.

Evidence Summary

Health professionals have long considered exposure to tobacco smoke harmful to reproduction, affecting aspects from fertility to fetal and child development and pregnancy outcome. Tobacco smoke contains thousands of compounds, some of which are known toxicants to reproductive health, such as CO, nicotine, and metals. About 10 percent of couples who want to conceive a child experience infertility or reduced fertility, approximately 10 to 20 percent of women who do conceive have miscarriage or stillbirth before delivery, and others have pregnancy complications and adverse outcomes that affect infant health and survival (CDC 2005).

In 2007, 17.4 percent of women and approximately 19 percent of women of reproductive age (18 through 44 years) smoked cigarettes (CDC 2008). From 2002 to 2005, 17.3 percent of pregnant women reported smoking cigarettes in the past month (*NSDUH Report* 2007). Because smoking rates have declined, persons involuntarily exposed to tobacco smoke probably now outnumber active smokers and they are exposed to some of the same toxins to which smokers are exposed.

Previous Surgeon General's reports and subsequent studies have found that smoking is related to several reproductive health endpoints. The 2001 report on women and smoking and the 2004 report on the health consequences of smoking noted a causal link between smoking and reduced fertility in women. Smoking may contribute to reduced fertility and other related reproductive endpoints, including earlier menopause or altered menstrual cycle parameters, through similar mechanisms such as by producing alterations in hormone function. Study findings suggest effects of smoking on estrogen and other hormones, which may vary by gender and the stage of life. Researchers have suggested that smoking has antiestrogenic effects, but more recent data are less consistent, at least for nonpregnant, premenopausal women. Studies implicate smoking and its effects on other hormones, such as progesterone, gonadotropins (FSH), and androgens (including in men). In animal studies, cells treated with alkaloids found in tobacco smoke, including nicotine, showed a dose-dependent inhibition of progesterone production, whereas estradiol production showed little effect or was slightly stimulated (Bódis et al. 1997; Gocze et al. 1999; Gocze and Freeman 2000; Miceli et al. 2005). Other scientists concluded that nicotine affects the menstrual cycle by inhibiting progesterone release just after ovulation. In animal models, nicotine acts on the HPG

axis, which is involved in normal sexual development and control of reproductive function (Matta et al. 1998). Studies show that prenatal exposure to nicotine is related to decreased testosterone levels in adult male rats and that cotinine, but not nicotine, inhibits testosterone synthesis in testes of neonatal rats (Sarasin et al. 2003).

Spermatogenesis is also affected by the hormonal milieu. Recent studies have provided more evidence that tobacco smoke exposure is associated with reduced sperm quality, which, in turn, may be a mechanism leading to reduced fertility in couples. Smoking-associated DNA damage in sperm could also be related to birth defects in offspring or producing nonviable gametes, resulting in apparent infertility or early fetal loss.

Many epidemiologic studies have related maternal smoking to reproductive problems that can originate in the oviduct (e.g., fallopian tube) (Stillman et al. 1986; Buck et al. 1997), including infertility and ectopic pregnancy. Studies have shown that exposure to tobacco smoke diminishes oviductal functioning. Exposure to cigarette smoke and its components has been shown to alter contraction in human and rabbit oviducts (Neri and Eckerling 1969; Ruckebusch 1975) and to form blebs on the oviductal epithelium in hamsters (Magers et al. 1995). In other studies of hamster oviducts, effects included decreased smooth muscle contractions and changed the ratio of ciliated to secretory cells, both of which would affect transit time of the egg through the oviduct (Huang et al. 1997; Knoll and Talbot 1998; DiCarlantonio and Talbot 1999; Riveles et al. 2003). Adhesion between the extracellular matrix of the oocyte cumulus complex and the tips of the cilia is essential for pickup of the oocyte and transport through the oviduct (Talbot et al. 1999; Lam et al. 2000). Exposure to both mainstream and sidestream smoke increases adhesion, which could account for decreased pickup rates even when cilia beat at normal or accelerated rates.

Ectopic pregnancy occurs when a fertilized egg is implanted outside the uterus, usually in the fallopian tube; it is estimated to occur in 1 to 2 percent of pregnancies. This condition accounts for approximately 6 percent of pregnancy-related deaths in the United States (Chow et al. 1987; Goldner et al. 1993; Berg et al. 2003; Chang et al. 2003; Van Den Eeden et al. 2005). Affected women are at increased risk of subsequent infertility and recurrent ectopic pregnancy, as would be expected among women with tubal damage (Chow et al. 1987; Coste et al. 1991; Washington and Katz 1993; Skjeldestad et al. 1998). The 2004 Surgeon General's report found the evidence suggestive but not sufficient to infer a causal relationship between smoking and ectopic pregnancy (USDHHS 2004) on the basis of a number of studies with significant associations between smoking and increased risk of ectopic pregnancy, yielding a pooled OR of 1.8 or an 80-percent increase with smoking (Castles et al. 1999). Two subsequent methodologically strong studies also indicate an increased risk with dose-response effects, further strengthening the evidence.

Studies have found evidence of a dose-response relationship even after adjustment for important potential confounders such as a history of sexually transmitted diseases and infertility (Bouyer et al. 2003; Karaer et al. 2006). In addition, plausible mechanisms for a relationship between smoking and ectopic pregnancy exist. As noted, the oviduct plays a critical role in the pickup and transport of the oocyte, and failure of this function can result in ectopic pregnancy (Talbot and Riveles 2005).

Spontaneous abortion, the involuntary termination of an intrauterine pregnancy before 20 weeks of gestation, has been reported in approximately 12 percent of recognized pregnancies; the majority occur before 12 weeks of gestation. Including very early pregnancy loss that may be unreported suggests an estimated 30 to 45 percent of conceptions actually end in pregnancy loss, some before implantation (Wilcox et al. 1988; Eskenazi et al. 1995). In addition to fetal abnormalities, other factors that likely contribute to SAB include maternal anatomic abnormalities of the uterus, immunologic disturbances, thrombotic disorders, and endocrine abnormalities (Christianson 1979; Cramer and Wise 2000; Regan and Rai 2000), some of which are affected by tobacco smoke. The 2004 Surgeon General's report (USDHHS 2004) found the evidence suggestive but not sufficient to infer a causal relationship between tobacco and SAB. Additional studies have shown positive associations, including two with measurement of cotinine (Ness et al. 1999; George et al. 2006) and two showing associations with secondhand smoke (Venners et al. 2004; George et al. 2006). Proposed mechanisms for an effect from tobacco smoke include vasoconstrictive and antimetabolic effects resulting in placental insufficiency and the subsequent demise of the embryo or fetus, fetal hypoxia from CO exposure, and direct toxic effects of cigarette smoke constituents.

Preeclampsia, marked by proteinuria, hypertension, and dysfunction of the cells lining the uterus, is a leading cause of pregnancy-related mortality in the United States (Berg et al. 2003). The 2004 Surgeon General's report found the evidence sufficient to infer a causal relationship between smoking and a reduced risk of preeclampsia (USDHHS 2004). Smoking has been proposed to reduce the risk of preeclampsia by effects of exposure to thiocyanate, which has a hypotensive effect (Andrews 1973), by changing the thromboxane A_2 to prostacyclin ratio, which would alter the way blood vessels constrict and dilate (Ylikorkala et al. 1985; Davis et al. 1987; Marcoux et al. 1989; Lindqvist and Maršál 1999), or by stimulating the

growth of new blood vessels in the placenta (Maynard et al. 2003; Fisher 2004; Jeyabalan et al. 2008).

Numerous studies have demonstrated the immediate effect of smoking one or two cigarettes on maternal heart rate and blood pressure (Lindblad et al. 1988; Morrow et al. 1988; Castro et al. 1993; Kimya et al. 1998; Ates et al. 2004). The clinical significance of a transiently elevated maternal heart rate on pregnancy is unknown. Studies that examined the acute effects of smoking after abstinence reported a transient increase in diastolic blood pressure and, to a lesser extent, systolic blood pressure. The release of catecholamine may trigger the elevations in maternal heart rate and blood pressure reported in these studies. However, smoking was also associated with an acute rise in plasma levels of norepinephrine, epinephrine, and dopamine, which could mediate the rise in maternal heart rate and blood pressure (Quigley et al. 1979). The literature indicates a transient increase in fetal heart rate with acute maternal smoking that was not statistically significant, although fetal heart rate variability, used to assess fetal well-being, was significantly transiently decreased (Graca et al. 1991; Ates et al. 2004). The clinical significance of these transient changes is not known. Studies on uterine and placental blood flow did not indicate basal differences between smokers and nonsmokers and results were inconsistent with respect to acute changes from smoking, so this mechanism is not supported as a possible cause of the well-documented fetal growth retardation among smokers.

Maintenance of pregnancy and fetal growth and development are dependent on normal formation of the placenta for exchange of nutrients and metabolic products between the mother and fetus. Studies have consistently shown that maternal smoking is associated with a thickening of the villous membrane of the placenta, which would decrease the ability of nutrients to pass through the placenta by diffusion (Burton et al. 1989; Jauniaux and Burton 1992; Demir et al. 1994; Bush et al. 2000; Larsen et al. 2002). The thickening of the villous membrane could contribute to fetal growth restriction. Researchers have hypothesized that toxic effects of maternal smoking on the placenta are responsible for the thickening of the villous membrane, perhaps caused by the accumulation of cadmium that is associated with a reduction in fetal capillary volume (Burton et al. 1989; Bush et al. 2000). Smoking appears to interfere with transformation of uterine spiral arteries, which is critical for the formation of the high-capacitance system that allows increased blood flow from the mother to the fetus. Such interference could lead to increased risk of adverse pregnancy outcomes such as miscarriage.

Epidemiologic studies have consistently found an increased risk of partial obstruction of the cervix by the placenta (placenta previa) among maternal smokers, which may lead to preterm delivery or maternal or fetal or neonatal death (Meyer et al. 1976; Zhang and Fried 1992; Monica and Lilja 1995). One mechanism commonly proposed to explain this association is the lower blood levels of O_2 and reduced blood flow that result from smoking, with compensatory placental enlargement.

Premature separation of the placenta from the uterus (placental abruption) increases the risk of perinatal mortality, and although several factors are involved (Misra and Ananth 1999), studies have consistently associated smoking with an increased risk (Raymond and Mills 1993; Ananth et al. 1999; Castles et al. 1999; Andres and Day 2000). The 2004 Surgeon General's report found the evidence sufficient to infer a causal relationship (USDHHS 2004), and researchers have observed a dose-response relationship (Raymond and Mills 1993; Ananth et al. 1999). One researcher has hypothesized that smoking-related degenerative and/or inflammatory changes in the placenta are factors related to abruption (Cnattingius 2004). Other hypotheses include low levels of vitamin C in maternal smokers (Faruque et al. 1995), leading to impaired collagen synthesis (Cnattingius 2004), microinfarcts, and accumulation of fatty deposits in placental vessels (Naeye 1979; Andres and Day 2000).

The 2004 Surgeon General's report found that the evidence was sufficient to infer a causal relationship between smoking and preterm delivery, a leading cause of neonatal morbidity and mortality in developed countries (USDHHS 2004). Smoking appears to increase the risk of both medically indicated and spontaneous preterm delivery (Kyrklund-Blomberg and Cnattingius 1998). Mechanisms through which smoking may contribute to preterm delivery include placental abruption or effects on the integrity of collagen, leading to a weakening and rupture of the membranes and increased risk of infections.

The same Surgeon General's report found sufficient evidence to infer a causal relationship between smoking and preterm premature rupture of membranes (PPROM) (USDHHS 2004). Researchers have hypothesized that smoking increases the risk of PPROM through several pathways. The effects of smoking on the immune system could increase the risk of genital tract infection or disrupt the cytokine system (French and McGregor 1996). Smoking could also increase the inflammatory response and reduce the availability of nutrients such as vitamin C or decrease the uptake of nutrients by the placenta (French and McGregor 1996; Lykkesfeldt et al. 1996, 2000), which would affect collagen content and membrane structure.

Maternal smoking has also been investigated for an association with birth defects, and although the 2004 Surgeon General's report (USDHHS 2004) found inadequate evidence for birth defects in general, maternal smoking

was considered suggestive for oral clefts. Evidence accumulated since the studies in that report is even stronger in supporting an association with smoking and cleft lip and/or palate (Little et al. 2004a), with some dose-response effects noted (Little et al. 2004b). Animal studies support these findings with evidence that nicotine can alter embryonic movements that are important during embryogenesis for the differentiation and maturation of organ systems, including palate closure (Panter et al. 2000; Ejaz et al. 2005). Other possible mechanisms include smoking-induced reductions in supply of essential nutrients for embryonic tissues, such as multivitamins and folate, fetal hypoxia from CO, or DNA damage from PAHs.

Deficiencies of micronutrients may contribute to adverse pregnancy outcomes, and smoking may contribute to this relationship. Vitamin C is required for collagen formation in amnion epithelial cells, and studies have noted reduced vitamin C levels in women with PPROM (Wideman et al. 1964; Casanueva et al. 1993). A decrease in the amount of collagen III likely leads to a weakening of the tensile strength of amniotic membranes, which could increase the risk of PPROM. Studies consistently show that smokers consume less and metabolize more vitamin C than do nonsmokers and thus have lower levels of vitamin C in plasma (Preston 1991; Lykkesfeldt et al. 2000; Cogswell et al. 2003) and in amniotic fluid (Barrett et al. 1991). Vitamin C is important for normal immune functioning, and a vitamin C deficiency in maternal smokers could contribute to adverse pregnancy outcomes by impairing maternal immune responses to genital tract infections. Because the placenta is impermeable to most proteins, proteins are usually synthesized by the fetus from amino acids supplied by the mother. Abnormalities in amino acid transport across the placenta can contribute to impaired fetal growth. Compared with nonsmokers, smokers have reduced levels of several amino acids in fetal plasma, umbilical plasma, and placental villi (Jauniaux et al. 1999, 2001).

CO forms as a by-product of combustion and is the toxin found in the highest concentration in cigarette smoke—10 to 20 times the dose of nicotine (Hoffman et al. 1997). The toxic effects of CO result predominantly from its binding to hemoglobin (Longo 1976, 1977), which is more than 200 times that of O_2 to hemoglobin (Hsia 1998). Fetal hemoglobin binds CO more tightly than does adult hemoglobin, so the fetus of a smoking mother has much higher levels of carboxyhemoglobin than those of the mother. The fetus experiences chronic hypoxia of fetal tissue, which persists for five or six hours of maternal smoking abstinence, such as during sleeping (Cole et al. 1972; Longo 1977; Bureau et al. 1982). CO from cigarette smoke deprives the fetus of O_2, which is essential for the aerobic metabolism that produces ATP, a coenzyme that stores the energy to fuel cellular activity throughout the body. Chronic mild O_2 deprivation in the fetus is likely a major underlying mechanism of smoking-associated fetal growth retardation, one of the most consistent and earliest identified adverse effects in infants of maternal smokers (Longo 1976, 1977; USDHHS 1980, 2004). Furthermore, infants of maternal smokers showed a decrease in birth weight nearly five times lower than that in infants of snuff users, even after adjustment for variables, implicating CO as the likely hazard (Longo 1976, 1977; England et al. 2003). Reduced fetal growth is associated with adverse effects on other endpoints, including perinatal mortality, birth defects, and neurodevelopment, which may also be directly affected by CO exposure.

Other components of tobacco smoke, including nicotine (see Chapter 3, "Chemistry and Toxicology of Cigarette Smoke and Biomarkers of Exposure and Harm"); heavy metals such as cadmium, lead, and mercury; and PAHs have been found to be causally associated with several adverse reproductive outcomes described here. Furthermore, levels of these components are found to be higher in smokers than in nonsmokers. It is more difficult to determine whether the levels generated from tobacco smoke are sufficient to cause these outcomes, but the evidence is suggestive for several of the components. The metabolism of these toxic constituents likely varies by genetic polymorphisms that reflect differential induction of drug- or toxin-metabolizing enzymes, such as cytochrome P-450s and GSTs, which, in turn, may modify the relationship between maternal smoking and birth outcomes, such as LBW or congenital anomalies. Genetic polymorphisms coding for the expression of tissue damage, inflammatory response, and immune mediator enzymes have been examined for interaction with maternal smoking in several studies of oral facial clefts, with *TGF*α and *TGFβ3* alleles most often implicated.

Conclusions

1. There is consistent evidence that links smoking in men to chromosome changes or DNA damage in sperm (germ cells), affecting male fertility, pregnancy viability, and anomalies in offspring.

2. There is consistent evidence for association of periconceptional smoking to cleft lip with or without cleft palate.

3. There is consistent evidence that increases in follicle-stimulating hormone levels and decreases in estrogen and progesterone are associated with cigarette smoking in women, at least in part due to effects of nicotine on the endocrine system.

4. There is consistent evidence that maternal smoking leads to transient increases in maternal heart rate and blood pressure (primarily diastolic), probably mediated by the release of norepinephrine and epinephrine into the circulatory system.

5. There is consistent evidence that links maternal smoking to interference in the physiological transformation of spiral arteries and thickening of the villous membrane in forming the placenta; placental problems could lead to fetal loss, preterm delivery, or low birth weight.

6. There is consistent evidence of the presence of histopathologic changes in the fetus from maternal smoking, particularly in the lung and brain.

7. There is consistent evidence that suggests smoking leads to immunosuppressive effects, including dysregulation of the inflammatory response, that may lead to miscarriage and preterm delivery.

8. There is consistent evidence that suggests a role for polycyclic aromatic hydrocarbons from tobacco smoke in the adverse effects of maternal smoking on a variety of reproductive and developmental endpoints.

9. There is consistent evidence that tobacco smoke exposure leads to diminished oviductal functioning, which could impair fertilization.

10. There is consistent evidence that links prenatal smoke exposure and genetic variations in metabolizing enzymes such as GSTT1 with increased risk of adverse pregnancy outcomes such as lowered birth weight and reduced gestation.

11. There is consistent evidence that genetic polymorphisms, such as variants in transforming growth factor-alpha, modify the risks of oral clefting in offspring related to maternal smoking.

12. There is consistent evidence that carbon monoxide leads to birth weight deficits and may play a role in neurologic deficits (cognitive and neurobehavioral endpoints) in the offspring of smokers.

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