Karolinska Institutet Danderyd Hospital
Division of Obstetrics and Gynaecology

SMOKING AND PREGNANCY,
with special reference to preterm birth and the feto-placental unit

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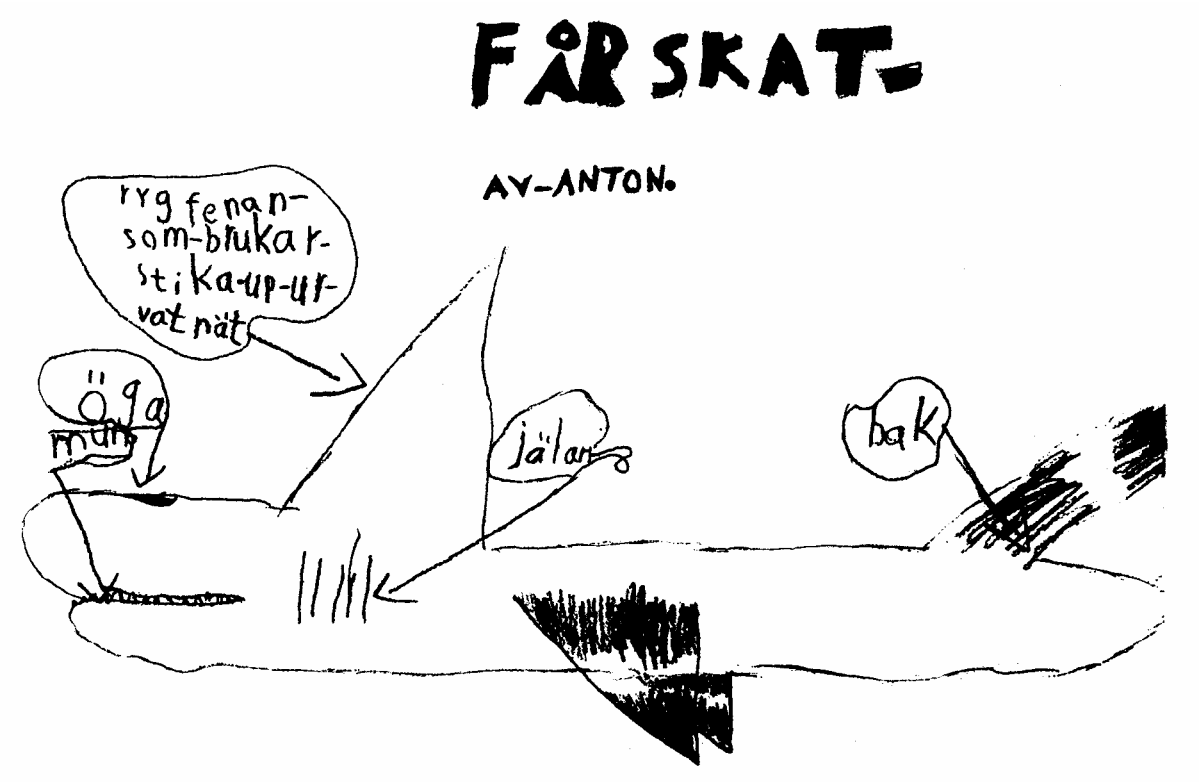
Where is the question to which human life is an answer?
(Jag söker den fråga på vilken människolivet är ett svar)
Willy Kyrklund “The Master Ma” 1953

Front page: engraving by Birgitta Kyrklund

To my Family



”Reeesearch” drawing by Anton 7 years

SMOKING AND PREGNANCY
with special reference to preterm birth and the feto-placental unit

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ABSTRACT

Objective: To study maternal smoking in pregnancy in relation to preterm birth, placental abruption and perinatal mortality in pregnancies with placental abruption, and to pulse wave characteristics in fetal aorta.

Methods: Two cohort studies with data on single births obtained from the Swedish Medical Birth Registry (N=311 977 and N=795 459, respectively). A case control study of very preterm birth (including 295 smokers and 295 non-smokers, respectively), with information retrieved from patient records. A clinical study of 34 smokers and 34 non-smokers in gestational age 31 to 40 weeks, where pulse wave measurements in the fetal aorta were made with an echo-tracking ultrasonic equipment. Pulse wave characteristics were analysed in relation to gestational age and smoking habits

Results: There was a dose-dependent relation between maternal smoking and preterm birth (<37 gestational weeks), both in very preterm (≤32 gestational weeks) and moderately preterm births (33-36 gestational weeks). Exclusion of pregnancies with smoking-related pregnancy complications did not essentially change the result. The association was stronger in spontaneous births compared to induced births. In very preterm birth, maternal smoking was a dose-dependent risk factor for preterm labour and probably also for 'idiopathic' labour (i.e., after excluding cases with infection, conisation of the cervix, hydramniosis, major uterine and fetal anomalies). Maternal smoking dose-dependently increased the risk of very preterm birth caused by late pregnancy bleedings (placenta praevia and placental abruption), and probably also of very preterm birth caused by preterm premature rupture of membranes. Maternal smoking was a dose-dependent risk factor for placental abruption and for perinatal deaths in pregnancies with placental abruption. For perinatal death, the risk was slightly higher in term births compared to preterm births. During gestation, pulse wave velocity increased in smokers but not in non-smokers. Mean incremental velocity did not change during gestation in smokers, but increased in non-smokers.

Conclusions: The studies demonstrated the fact that maternal smoking is a modest risk factor for preterm birth, is a risk factor for spontaneous labour in very preterm birth, and is a major risk factor for placental abruption and perinatal death in pregnancies with placental abruption. The finding of alterations of pulse wave characteristics during gestation may be a sign of increased vessel stiffness in smokers, and an indication of a possible influence of maternal chronic smoking on the feto-placental circulation. The results emphasise the need of further campaigns against smoking among women.

Key words: Smoking, Preterm birth, Very preterm birth, Onset of delivery, Placental abruption, Perinatal death, Pulse wave, Vessel stiffness, Echo-tracking system.

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LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by the Roman numerals.

I. Nina B. Kyrklund Blomberg, Sven Cnattingius. Preterm birth and maternal smoking: risks related to gestational age and onset of delivery. Am J Obstet Gynecol 1998;179:1051-5.

II. Nina B. Kyrklund-Blomberg, Fredrik Granath, Sven Cnattingius. Maternal smoking and causes of very preterm birth. Acta Obstet Gynecol Scand 2005;84:572-77.

III. Nina B. Kyrklund-Blomberg, Gerhard Gennser, Sven Cnattingius. Placental abruption and perinatal death. Paediatr Perinat Epidemiol 2001;15:290-297.

IV. Nina B. Kyrklund-Blomberg, Jie Hu, Gerhard Gennser. Chronic effects of maternal smoking on pulse waves in fetal aorta. (submitted)

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ABBREVIATIONS AND DEFINITIONS

Preterm birth	Delivery at a gestational age less than 37 gestational weeks
Very preterm birth	Delivery at a gestational age of not more than 32 gestational weeks
Moderately preterm birth	Delivery at a gestational age of more than 32 gestational weeks and less than 37 gestational weeks
LBW	Low birth weight Birth weight of less than 2500 grams
PROM	Premature rupture of membranes Rupture of fetal membranes 24 hours before start of delivery contractions
IUGR	Intra uterine growth retardation Ultrasonically estimated size less than 2 standard deviations below the mean size
SGA	Small for gestational age Birth weight less than 2 standard deviations below the mean birth weight for gestational age.
Stillbirth	Fetal death at 28^{th} gestational week or later
Early neoanatal death	Live born infant's death during the first 7 days of life
Perinatal death	Stillbirth and early neonatal death
SIDS	Sudden infant death syndrome
Parity	Previous births including stillbirths plus current birth
Not smoking	No daily smoking
Moderate smoker	1-10 cigarettes per day
Heavy smoker	More than 10 cigarrettes per day
MBR	Medical Birth Registry (Medicinska Födelseregistret)
ICD	The International Classification of Diseases of the World Health Organisation
OR	Odds ratio
CI	Confidence interval
Dd	Minimum diameter in diastole
ΔD	Pulse amplitude
Strain	ΔD /Dd, The ratio of the deformation to the original form
PWV	Pulse wave velocity, the distance the pulse wave travels in a certain time
MIV	Maximum incremental velocity, the maximum first derivative of pulse wave upstroke

INTRODUCTION

Smoking tobacco is highly addictive and harmful for nearly every organ in the body, as well as for fertility, pregnancy and pregnancy outcome. Although smoking is declining in the western countries, it is increasing among women in many other parts of the world. Smoking has an influence on preterm birth and placental abruption, and also causes placental pathology and an immediate fetal vascular response. Preterm birth is a major cause of perinatal mortality and morbidity. Placental abruption is less common but a threatening disorder for mother and child, with a high maternal and perinatal mortality rate. Intervention programmes have not been effective, and there are reports on rising rates of preterm birth and placental abruption. The relation of fetal environment to health later in life, specifically the role of fetal growth restriction, has come into focus during the last decade through the Barker hypothesis (Barker, Winter et al. 1989). In this respect, the impact of maternal smoking on the feto-placental vascular unit is of great interest.

In the present thesis, the impact of maternal smoking has been studied from various aspects. A large register study of maternal smoking as a risk factor for preterm birth in relation to gestational age and onset of labour was performed. This was followed by a study of very preterm birth, conducted by means of information from patient records, in order to clarify the role of maternal smoking in relation to causes of very preterm birth. Another large register study was made on risk factors for placental abruption, and perinatal death in pregnancies with placental abruption. Finally, a prospective clinical study on pulse wave characteristics in the fetal aorta in relation to daily maternal smoking was carried out.

BACKGROUND

Smoking

History of tobacco

The Indians had used tobacco for religious and ceremonial causes for hundreds of years when Christopher Columbus came to the West Indies in 1492. The Arawak Indians introduced them to the use of tobacco, and in 1560 Jean Nicot, the French ambassador in Lisbon, brought the habit of smoking and snuffing to Europe. According to old customs accounts tobacco was introduced in Sweden in the beginning of the 17th century. Soon, farming and production of tobacco started in Sweden, and in 1724 there was an order from the king that every city should reserve land for cultivation of tobacco. The last tobacco farm in Sweden closed in 1964. In the 19th century there were more than a hundred industrial plants for tobacco products, mainly in southern Sweden. Snuff is still produced in several places in Sweden, but the last factory for cigarette production was closed in 2002 (FHI 2005).

Tobacco and public health

Smoking is the most important single risk factor for diseases and death in Sweden, and is one of the major causes of too early death in the industrialized part of the world. The World Health Organization (WHO) has estimated that smoking globally each year causes about 5 millions too early deaths. If the present trend lasts, it will increase to 10 millions during the next 20 years, seven millions of which will be in developing countries. About 50% of the smokers loose on average 7-8 years of their expected lifetime. Today, about half the deaths caused by tobacco strikes middle age people, mainly men, who loose on average 22 years. In Sweden, the number of too early deaths because of active smoking is estimated to around 6,400 per year, and additionally about 500 persons die due to environmental smoking (WHO 1999; FHI 2005).

Since the 1960's, more than 60 000 scientific papers about the health risks of smoking have been published (FHI 2005). Smoking damages nearly every organ in the body. Major risks of smoking are lung cancer, other forms of cancer, cardiovascular diseases and chronic obstructive pulmonary disease. It is estimated that smoking causes 90% of all lung cancer and 20% of the cardiovascular diseases (FHI 2005).

Smoking is also a risk factor for peptic ulcers, respiratory infections, osteoporosis, periodontitis, cataract, impotence, complications after surgery, and it also affects fertility and pregnancy (Sgr 2004). Smokeless tobacco is also harmful, and seems to increase the risks of oral and pancreatic cancer, to impair the endothelium and in pregnancy it seems to be harmful for the fetus (England, Levine et al. 2003; FHI 2005).

In women, smoking is associated with cervical and vulvar cancer, but the major cancer risk is lung cancer – the same as in men. For women younger than 50 years, the majority of cardiovascular disease is attributed to smoking. Smoking women who use hormonal contraceptives have a particularly elevated risk of cardiovascular disease. Evidence is conflicting concerning the risk for cardiovascular disease in smokers using hormonal replacement therapy. Risk is also elevated for ischemic stroke, subarachnoid haemorrhage, carotid and peripheral atherosclerosis and aortic aneurysms. Smoking causes oestrogen-deficiency disorders, earlier menopause, a more masculine fat distribution and osteoporosis (Sgr 2001). Between 1950 and 2000, about ten million women died from tobacco use, and it is estimated that tobacco-attributable deaths among women will more than double over the next

30 years (Jacobs 2001). Cohort studies have shown that the annual risk for death is almost doubled in smoking women compared to those women who have never smoked (Sgr 2001).

Smoking and pregnancy risks

In smoking women there is risk for conception delay, primary and secondary infertility, and probably for spontaneous abortions and ectopic pregnancy. During pregnancy there are elevated risk for placental abruption, placenta praevia, premature rupture of membranes (PROM), preterm birth and a decreased risks of pregnancy-induced hypertensive diseases (gestational hypertension and preeclampsia). Outcome risks in pregnancy are stillbirth, neonatal death, decrease in birth weight, small for gestational age (SGA), sudden infant death syndrome (SIDS), and disturbed lactation, whereas data on neurocognitive development and risks of childhood cancer are inconsistent (Fredricsson and Gilljam 1992; Sgr 2001; Cnattingius 2004). In a survey of female health employers in the US, 91% had knowledge about the increased risk of complications in pregnancy, but only a minority knew about the risks of miscarriage, infertility and ectopic pregnancy (Roth and Taylor 2001).

Prevalence of smoking

In Sweden, in 1946 about 50% of the men and 9% of the women were smokers. In 1963 the rate of smoking had not changed among men, but in women it had increased to 23%. Since then, the smoking prevalence started to decrease among men. Among women, the smoking prevalence increased until a top of 32% in the 1970's and has hereafter steadily declined (FHI 2005). In 1980, 36% of the men and 29% of the women were daily smokers. In 2004, the prevalence of smoking was 14% among men and 19% among women (FHI 2005). Since 1994, women in Sweden have been smoking more than men, although the prevalence is declining in both groups. The smoking prevalence is higher among those with a low educational level and poor socio-economic circumstances, which strikes women especially hard (Bostock 2003). For example, among women in the US, daily smoking is three times as common among those with lower education (9 to11 years) compared with those with higher education (more than 16 years) (Sgr 2001).

Prevalence of smoking in pregnancy

In Sweden, smoking prevalence in early pregnancy has decreased from 31% in 1983 to 11% in 2002. Those who get pregnant reduce their tobacco use substantially. In Sweden about 50% quit smoking before their first visit to the antenatal clinic (SoS 2005). Smoking is more prevalent in young mothers. For example in Sweden, in 2002 more than 50% of pregnant teenagers smoked three months before pregnancy, whereas the corresponding rates at 20 to 24 years were 37%, and at 25 to 29 years 21%, and at ages over 30 years 16 to17% (SoS 2005). In the US, 20% of the women were smoking during pregnancy in 1989, and 13% in 1998, and about 5% had stopped smoking when they learnt they were pregnant. Among women with a high educational level (16 years or more), 2% were smoking during pregnancy, while among those with low educational level (9 to11 years), 26% were smokers. Smoking prevalence was higher in white women than in black women (20% and 14% respectively in 1990)(Sgr 2001).

Smoking cessation and preventive measures

Sweden was the first country in the world where the prevalence of female smokers exceeded that of male smokers (FHI 2005). Although several effects of tobacco (hormonal and pregnancy related) are of special interest for women, tobacco control initiatives did not take the diversity of a population into account, and did not, until recently, specifically address women's concerns. The early epidemiologic research was carried out on men, since the devastating consequences of smoking were initially most prominent in men (Christofides 2001). In

Sweden, nationwide smoking cessation programmes have been carried out for about 25 years through collaborative efforts of the National Board of Public Health and Welfare (Socialstyrelsen), the Swedish Cancer Society (Cancerfonden), the Swedish Heart and Lung Foundation (Hjärt- och Lungfonden) and The National Institute of Public Health (Folkhälsoinstitutet), the latter being the main actor. The issue of information directed to women specifically, and not only to pregnant women has been increasingly recognized (Sgr 2001; WHO 2001; FHI 2005).

In Sweden, midwifes and child nurses inform about tobacco attributed risks of pregnancy complications and child health, and take remedial measures against use of tobacco at the visits to antenatal and child care clinics. The National Board of Health and Welfare in Sweden shows in their latest report, about smoking habits among pregnant women, that about 50% of smoking women who get pregnant quit their habit before the first visit to the antenatal clinic, but that in 2002, 10.6% of all pregnant women were still daily smokers (SoS 2005). At the first visit to the antenatal clinic 30.5% of the pregnant teenagers still smoked, and 18.5% of those 20 to 24 years of age, 9.4% of those 25 to 29 years of age, and at ages over 30 years about 8 to10% were daily smokers. Eight months after birth, 9.3% of the mothers and 13.3% of the fathers smoked (SoS 2005).

Also in the US a higher percentage quit smoking during pregnancy than other times in life. It is also reported that only about one third of the women who stop smoking during pregnancy are still abstinent one year after delivery (Sgr 2001). In reports from the US Surgeon Genereal and Cochrane reviews, smoking cessation programmes in pregnancy are considered to be cost-effective, reducing the proportion of women who continue to smoke, as well as the prevalence of low birth weight and preterm birth. However, the pooled trials have inadequate power to detect reductions in perinatal mortality and very low birth weight (Sgr 2001; Lumley, Oliver et al. 2004). In a Swedish cessation program, that was introduced to smokers at the first visit to the antenatal clinic, 10.4% of the participants quit smoking up to eight weeks after delivery, compared to 5.2% in the control group (Hjalmarson, Hahn et al. 1991).

Nicotine

Nicotine is a strongly addictive drug, which stimulates the reward system in the brain. Circulating levels of noradrenalin and adrenaline increase, and the bioavailability of dopamine is also altered. Nicotine also influences a number of hormones, including vasopressin, beta-endorphin, growth hormone and adrenocorticotropic hormone (ACTH) (Pomerleau 1992; Lambers and Clark 1996). Nicotine influences all aspects of the immune system, including alterations in humoral and cellular immunity (McAllister-Sistilli, Caggiula et al. 1998).

Nicotine has a half-life of 1 to 2 hours. It's primary metabolite, cotinine has a half-life of 15 to 20 hours and is therefore a better indicator of nicotine exposure. Maternal cotinine levels have been shown to be a stronger predictor of low birth weight (LBW) than self-reported use of tobacco (Haddow, Knight et al. 1987).

Nicotine is shown to have obvious effects on the fetus. Nicotine readily gains access to the fetal compartment, with fetal concentrations generally 15% higher than maternal levels, and it concentrates in fetal blood, amniotic fluid and breast milk (Lambers and Clark 1996). Maternal intravenous nicotine administration in term pregnant sheep produced significant increases in fetal arterial blood pressure and umbilical vascular resistance, decreased fetal heart rate, and umbilical blood flow, but did not significantly alter umbilical systolic/diastolic (S/D) ratios. Maternal effects included increased blood pressure and heart rate (Clark and Irion 1992). One

should, however emphasize that when tobacco is smoked, apart from nicotine a great variety of other substances are inhaled. It is therefore of interest to note that smokeless tobacco (i.e., Swedish snuff), including predominantly nicotine, is shown to be a risk factor for preterm delivery, SGA, and preeclampsia (England, Levine et al. 2003).

Preterm birth

Preterm birth - Definitions

Preterm birth is, according to the WHO, the delivery of an infant before 37 weeks or 259 days of gestational age (WHO 1970), and LBW is traditionally defined as less than 2500 grams. Very preterm birth is defined as gestational age less than 32 weeks, and extremely preterm birth as less than 28 weeks (Moutquin 2003). Since the introduction of ultrasound screening programmes in the 1970's, with dating of the pregnancy, the possibility to identify those born preterm is good. In Sweden all pregnant women are offered a free routine ultrasound examination, and about 97% accept this offer (SBU 1996). In many countries, data on preterm birth still rely on information on the last menstrual period or even on LBW.

Many LBW infants are not preterm but rather born with a low birth weight for gestational age, i.e., they are SGA. In developed countries most of the perinatal morbidity and mortality associated to LBW is related to low gestational age at birth rather than to fetal growth retardation. Worldwide, however, fetal growth retardation is a greater problem than preterm birth (Villar and Belizan 1982).

Preterm birth - Trends

The prognosis for the preterm infant has improved substantially, predominantly because of improvements in neonatal care. In contrast, most intervention programmes, aiming at reducing the incidence of preterm birth have not been successful, and the rate of preterm birth continues to be about 5% to 10% of all births in western countries (Collaborative-Group 1993; Meis, Michielutte et al. 1995). In the US, the rate has even increased from 9.4% in 1981 to almost 12% in 2000, in spite of a decreasing rate among blacks who have a considerably higher incidence compared to whites (Creasy 1993; Goldenberg, Iams et al. 2003; Ananth, Joseph et al. 2005). Increasing rates of preterm birth is also shown in other countries, i.e., in Denmark (from 5.6% in 1994 to 6.9% in 2003) and in Canada (from 6.3% in 1981-83 to 6.8% in1992-94) (Joseph, Kramer et al. 1998). In Sweden there has, although multiple pregnancies have increased, been a decrease in preterm birth rates from 6.3% in the mid 1980s to 5.6% in 2001 (Morken, Kallen et al. 2005). In Finland rates of preterm birth in the late 1980's were 5.2%, increasing to 6.0% in 2000, which was followed by a decrease to 5.6% in 2004 (SoS 2005).

The very preterm births (≤32 weeks) account for about 1 to 2% of all births. An analysis of the falling trend of preterm births in Sweden, showed that this decrease was mainly caused by a reduction of singleton preterm births of gestational age 34 to 36 weeks (Morken, Kallen et al. 2005).

Preterm birth - Neonatal morbidity and mortality

Preterm birth is the major cause of neonatal morbidity and mortality, and these risks increase with decreasing gestational age. Probably more than 70% of the total perinatal mortality is due to preterm birth (Creasy 1993; Goldenberg 2002). There is a high risk of pulmonary distress, sepsis, necrotizing enterocolitis, cerebral haemorrhage, cerebral leukomalacia and cerebral palsy followed by impaired neuropsychological development (Keirse 1995; Finnstrom, Otterblad-Olausson et al. 1999; Stjernqvist and Svenningsen 1999). Preterm birth may also lead to long term health consequences. A study in the US of eight year old children with a birth weight <1000g showed significantly decreased functional abilities, academic skills, motor skills and adaptive functioning compared to children of normal birth weight (Hack, Taylor et al. 2005).

The shorter the gestational age, the smaller the rate of preterm birth, and the higher mortality and morbidity (Goldenberg and Rouse 1998). Neonatal mortality decreases dramatically with every additional week of gestational age, at 22 weeks less than 10% survive, at 23 weeks about 20%, at 24 weeks about 40%, at 25 weeks about 50%, at 26 weeks about 60%, at 27 to 30 weeks about 80 to 90% and at 32 weeks almost 100% (Slattery and Morrison 2002).

In the US, preterm birth is the leading cause of neonatal mortality (deaths during the first four weeks of life) in blacks, while in whites, preterm birth is the second cause after congenital anomalies (Carmichael, Iyasu et al. 1998; Mattison, Damus et al. 2001).

Preterm birth - Presentation of delivery

About two thirds of all preterm births are spontaneous, and 1/3 is electively delivered due to fetal or maternal reasons, and classified as induced, iatrogenous or indicated preterm birth (Arias and Tomich 1982; Meis, Michielutte et al. 1995). Among spontaneous preterm births, the majority present with preterm labour and intact membranes, and the rest with preterm premature rupture of membranes (preterm PROM) (Tucker, Goldenberg et al. 1991). Spontaneous preterm labour may be followed by spontaneous or operative delivery. There is often confusion about the classification: "spontaneous labour or preterm PROM?". According to the International Classification of Diseases (ICD), spontaneous labour includes deliveries where contractions start within 24 hours after rupture of the membranes. The risk of neurological damage and sequels is higher in spontaneous preterm birth, where connections to chorioamnionitis, elevated interleukines and damage of white matter in the brain are often found (Verma, Tejani et al. 1997; Martinez, Figueroa et al. 1998; Yoon, Romero et al. 2000).

Mechanisms of term labour

What is known about the mechanism of term and preterm labour is described in several reviews (Norwitz, Robinson et al. 1999; Challis and Smith 2001; Astle, Slater et al. 2003; Keelan, Blumenstein et al. 2003; Peltier 2003). During pregnancy, there is an alteration in the maternal immunity that prevents rejection of the fetal allograft. The uterus is maintained relaxed through a complex balance between inhibitors and stimulators in the three compartments involved, the fetus, the placenta/fetal membranes and the mother. Before start of labour, there is a period of priming or activation, with a rebuild of cervical tissue collagen and an increase in contraction associated proteins (CAP), i.e., gap junctions, oxytocin and prostaglandin receptors in the myometrium. In the shift from relaxation to stimulation of the myometrium to labour contractions, the fetal hypothalamic-pituitary-adrenal axis (HPA) is activated with a rise in dehydroepiandrostendione (DHEAS) and cortisol, resulting in a rise in placental cytokines, corticotropin-releasing hormone (CRH), oestrogen, prostaglandins, and oxytocin, and probably a functional decrease in progesterone. Post-delivery thrombin in the bleeding placental site is an activator for involution of the uterus. The trigger of labour is still unknown, but the activation of the myometrium is probably related to two separate but interdependent pathways, i.e. activation of the fetal HPA-axis and mechanical distension of uterus leading to an up regulation of CAP.

Mechanisms of preterm labour

In preterm labour, the balance of inhibition and activation of the uterus is disturbed and a cascade of activating processes initiate labour too early (Hagberg and Wennerholm 2000; Norwitz and Robinson 2001). Compared with term labour, the inflammatory activation in the fetal membranes and decidua is much more pronounced in preterm labour, particularly in the presence of infection (Keelan, Blumenstein et al. 2003). The following (not exclusive) main

pathways for initiation of preterm labour are discussed below: 1. infection/inflammation, 2. vascular pathology, 3. stress, 4. genetic differences, 5. mechanical stress (Mattison, Damus et al. 2001).

1. Infection/inflammation

Infection probably has a causal relationship to spontaneous preterm birth, and infection/inflammation is more common in spontaneous compared to induced birth (Goepfert, Andrews et al. 2004). About 20 to 40% of all preterm births are probably caused by infections, and infections increase with decreasing gestational age (Watts, Krohn et al. 1992; Andrews, Hauth et al. 2000; Challis, Lye et al. 2001).

The major part of infections contributing to preterm labour and delivery are intrauterine infections. These are probably mainly caused by bacteria ascending from the vagina through the cervix, although some cases may be due to microbial invasion of the uterine cavity secondary to preterm PROM or labour (Romero, Gomez et al. 2001). There may also be haematogenous dissemination of bacteria or inflammatory mediators (cytokines etc) through the placenta, since extra-uterine systemic maternal infections are also associated with preterm labour and delivery (Romero, Gomez et al. 2001).

Clinically silent intrauterine infections are more common than traditionally recognized, and histological chorioamnionitis is shown to have a strong association to preterm delivery (Salafia, Vogel et al. 1991; Arias, Rodriquez et al. 1993; Goldenberg, Hauth et al. 2000; Romero, Gomez et al. 2001; Yoon, Romero et al. 2001). In 33 studies where amniocentesis in preterm labour with intact membranes were performed, the mean rate of positive cultures (i.e., detected infections in amniotic fluid) was 12.8% (Romero, Gomez et al. 2001). Pregnancies with positive cultures did not generally have clinical evidence of infection. However, these pregnancies developed chorioamnionitis to a higher extent (37.5 vs 9%), they were more refractory to tocolysis (85.4 vs 16.3%), and had more preterm PROM (40 vs 3.8%) compared to those with negative amniotic cultures. The earlier gestational age at preterm delivery the more likely was a positive culture.

Inflammation and preterm birth also seem to appear without positive cultures. Antibiotic treatment is inefficient in most cases for treatment of preterm labour with intact membranes, but more efficient in cases of preterm PROM (Andrews, Hauth et al. 2000; Klein and Gibbs 2004). In term delivery the effect of the anti-inflammatory progesterone probably decreases, and positive results of progesterone supplementation for preventing preterm birth have been reported. This supports that non-bacterial inflammation may also be a part of the aetiology of preterm contractions (Astle, Slater et al. 2003; Elovitz and Wang 2004; Dodd, Crowther et al. 2005).

2.Vascular

An evident vascular collapse is placental abruption which is a well known cause of preterm birth (Ananth, Berkowitz et al. 1999). However, placental vascular deficiencies can also be manifested as smaller vaginal bleedings, often without other symptoms. Such bleedings are not always harmless, since women with first trimester bleedings are more prone to deliver preterm (Williams, Mittendorf et al. 1991). Signs of bleeding in the basal plate are related to histological evidence of chronic utero-placental vascular pathological processes. In cases of spontaneous preterm birth this may be associated with decidual bleeding, occasionally clinically manifested as gestational bleeding (Salafia, Lopez-Zeno et al. 1995). An association

of vascular lesions in the placenta to preterm birth is reported, and a relation to visible bleeding seems not to be necessary (Arias, Rodriquez et al. 1993; Salafia, Lopez-Zeno et al. 1995; Germain, Carvajal et al. 1999). Despite evidence that defective placentation is associated with spontaneous preterm delivery, Doppler measurements of uterine artery resistance in the second-trimester, is not different in pregnancies subsequently complicated by preterm labor compared to pregnancies delivered at term.(Cobian-Sanchez, Prefumo et al. 2004)

3. Stress, socioeconomic disparities, nutrition

Explored risk factors only account for about half of all preterm births. Thus, known risk factors have low sensitivity and specificity, which may partly explain why intervention programmes have not been successful (Wadhwa, Culhane et al. 2001). There is growing evidence that psychological, social and economic stress (including violence and racism) increase risks of preterm birth and perinatal mortality (Kiely and Susser 1992; Hogue, Hoffman et al. 2001; Kramer, Goulet et al. 2001; Moutquin 2003). Rates of preterm births in the US is almost the double compared to the rate in Sweden, and some explanatory factors might be the high attendance to antenatal care clinics, less disparity in socio-economic status and less racial conflicts in Sweden compared to in the US (Kramer, Goulet et al. 2001; Cnattingius 2004). Wadhwa et al have summarized the probable ways that stress act on the mechanism of preterm birth (Wadhwa, Culhane et al. 2001). It seems as if stress is working through interacting neuroendocrine and immuno-modulating pathways. It is postulated that stressor-induced elevations in cortisol and catecholamines alter the immune response and increase free placental CRH that may act as an uterotonic agent (Keelan, Coleman et al. 1997).

4. Genetical

Timing of delivery is believed to be in control of the fetus, and there is growing evidence that there is also a genetic pathway for preterm birth (Kramer, Goulet et al. 2001).Previous preterm birth is a strong risk factor for preterm birth, and also mothers who are born preterm seem to have an elevated risk of giving birth preterm (Bakketeig, Hoffman et al. 1979; Porter, Fraser et al. 1997). Results from a Swedish twin study suggest that genetic factors account for 30 to 40% of all preterm births (Clausson, Lichtenstein et al. 2000). Risk of very preterm birth is found to increase in mothers conceived by an elderly partner. Racial differences when it comes to preterm birth seem not to be fully explained by socio-economic factors (Goldenberg, Cliver et al. 1996; Zhu, Madsen et al. 2005). Recently, studies on exploring genes predisposing for preterm delivery have started and supportive results are published (Chen, Hu et al. 2004; Engel, Erichsen et al. 2005). The results suggest that common genetic variants in proinflammatory cytokine genes could influence the risk for spontaneous preterm birth, and that also genes affecting vascular function might increase the risk of preterm delivery.

5. Mechanical

Reviews over preterm birth often list uterine distension, uterine malformation and cervical incompetence as risk factors (Robinson, Regan et al. 2001; Haram, Mortensen et al. 2003; Moutquin 2003). Distension can be caused by multifetal pregnancy and by hydramniosis. Malformations are caused by uterine embryonic anomalies, fibroids and iatrogenic causes (surgery and diethylstilbestrol). Cervical incompetence can be caused by surgery, induced abortions, and by pharmaceutics (diethylstilbestrol).

Riskfactors of preterm birth

Information about risk factors listed in Table 1 is extracted from reviews of preterm birth (Hagberg and Wennerholm 2000; Mattison, Damus et al. 2001; Norwitz and Robinson 2001; Robinson, Regan et al. 2001; Slattery and Morrison 2002; Haram, Mortensen et al. 2003).

Table 1. Risk factors for preterm birth

Risk factors	Proposed biological pathways
Extragenital infections: i.e. urinary tract infections, pneumonia, periodontitis etc Infections ascending from the vagina: i.e. chorioamnionitis with positive culture, Group β-streptococcus, bacterial vaginosis etc Aseptic chorioamnionitis	Infection/Inflammation acts through increase of cytokines which initiate formation of prostaglandins Stress and genetic factors might increase the susceptibility through changes in the inflammatory response
Low socio-economic status Low level of education Physical exercise, Blue-collar work Low maternal weight Stressful events Domestic violence Racial insults No cohabitation with infants father Absent prenatal care	Stress acts mainly through fetal HPA-axis and increase of placental cortisol and CRH
Parity Age <20 or >40 years Infertility >3years Low prepregnancy weight Diabetes Early pregnancy bleeding Late pregnancy bleeding Hypertensive diseases Anaemia Poor nutrition Drugs Smoking	Vascular pathology may cause hemorrhage with release of trombin that activate prostaglandines may cause hypoxia and inflammation Vascular pathology and inflammation might occur by environmental causes
Race History of preterm birth History of late spontaneous abortions	Stress Genetic Infection/inflammation
Multifetal pregnancy Hydramniosis Uterine/cervical malformation Conisation of the cervix Induced abortion	Mechanic Stretch activates prostaglandines Surgery may have decreased the normal durability of uterus or cervix

Placental abruption

Definition

Placental abruption is a too early separation of the placenta from the uterine wall. There can be a total separation, which in most cases causes fetal death. A partial separation can be more difficult to diagnose, especially if it is small. Usually a combination of diagnostic criteria constitutes the base for diagnose: antepartum haemorrhage, blood clot or impression behind the placenta, histological evidence, pain, and signs of asphyxia. Sonography is generally not sensitive for detection of placental abruption, but a positive finding is reported to be associated with worse neonatal outcome (Glantz and Purnell 2002).

Trends

Placental abruption is a rare but life-threatening complication in pregnancy that occurs in about 0,4% to 2% of all pregnancies, and has a perinatal mortality of around 10-20% (Karegard and Gennser 1986; Raymond and Mills 1993; Ananth, Oyelese et al. 2005; Salihu, Bekan et al. 2005). There are no intervention programmes for prevention of placental abruption and there are reports on increasing rates (Saftlas, Olson et al. 1991; Rasmussen, Irgens et al. 1996). In the US, the increasing prevalence of placental abruption is reported to be stronger among black compared to white women: From 1979-1981 to1999-2001 the prevalence of placental abruption increased from 0.76% to 1.43% in black women, whereas the corresponding increase in white women was from 0,88% to 0.94% (Ananth, Oyelese et al. 2005). In white women, the increasing prevalence of placental abruption was attributed to increased rates of preterm labour and diabetes.

Risk factors

Well-established maternal risk factors for placental abruption are high age (>35 years), parity, afro-american race, smoking, drug abuse, and low education. In addition, also other socioeconomic disadvantages, recent abdominal trauma and poor prenatal care are reported to increase the risk of placental abruption (Raymond and Mills 1993; Spinillo, Capuzzo et al. 1994; Kramer, Usher et al. 1997; Ananth, Berkowitz et al. 1999; Salihu, Bekan et al. 2005). Maternal medical determinants of placental abruption are diabetes, chronic hypertension, anaemia and fibroids (Spinillo, Capuzzo et al. 1994; Ananth, Savitz et al. 1996; Ananth, Oyelese et al. 2005).

Pregnancy complications consistently associated with risk of placental abruption are pregnancy-induced hypertensive diseases, SGA, multifetal pregnancies, preterm PROM and preterm labour (Raymond and Mills 1993; Kramer, Usher et al. 1997; Sheiner, Shoham-Vardi et al. 2002; Sheiner, Shoham-Vardi et al. 2003; Ananth, Oyelese et al. 2005). Risk of placental abruption is also related to bleedings in the first and second trimester, chorioamnionitis, short umbilical chord, velamentous chord insertion, (Sipila, Hartikainen-Sorri et al. 1992; Weiss, Malone et al. 2004; Ananth, Oyelese et al. 2005) and fetal characteristics such as hydramniosis, congenital heart anomalies, and male fetal gender (Saftlas, Olson et al. 1991).

Complications in previous pregnancies are also associated to placental abruption. A history of previous placental abruption is a strong risk factor, but the risk is also increased in women with a previous Caesarean delivery, a previous preterm birth, a previous SGA, prior pregnancy induced hypertension, and among women with a history of infertility or recurrent spontaneous abortions (Karegard and Gennser 1986; Ananth, Savitz et al. 1996; Rasmussen, Irgens et al. 1999; Lydon-Rochelle, Holt et al. 2001; Pandian, Bhattacharya et al. 2001; Sheiner, Levy et al. 2005).

Besides high perinatal mortality, there are other increased outcome risks of placental abruption, including preterm birth, non-vertex presentation, sudden infant death syndrome (SIDS) and cerebral palsy (CP) (Naeye, Harkness et al. 1977; Klonoff-Cohen, Srinivasan et al. 2002; Sheiner, Shoham-Vardi et al. 2002; Matsuda, Maeda et al. 2003; Sheiner, Shoham-Vardi et al. 2003). Perinatal mortality in pregnancies complicated with placental abruption is strongly associated to preterm birth and SGA: about 50% of the deaths are reported to be due to early delivery. However, the high risk of perinatal mortality persists in all gestational ages after controlling for fetal growth retardation and preterm delivery (Ananth and Wilcox 2001). A strong determinant of perinatal death in pregnancies with placental abruption is smoking: Naeye and co-workers in 1980 reported about 50% reduction in rates of fetal and neonatal deaths due to placental abruption, in women who had stopped smoking at the first antenatal visit and did not relapse (Naeye 1980).

Mechanisms of placental abruption

When birth of the infant is completed, the delivery finishes by separation of placenta from the uterine wall and its expulsion from the uterine cavity. The mechanisms behind the initiation of this normal separation are unclear, and little is also known about the mechanisms behind the too early placental separation in placental abruption.

Bleeding causes a release in thrombin, which is a potent stimulus of uterine contractions, and probably contributes to the increased risks of preterm labour and preterm birth in pregnancies complicated with placental abruption (Elovitz, Ascher-Landsberg et al. 2000). The etiological determinants of placental abruption indicate that vascular placental impairment, probably already from early pregnancy, enhances the risk of placental abruption. Placental abruption may share etiological factors with pregnancy-induced hypertensive diseases, SGA and preterm birth. Alternatively, these complications may represent different expressions of recurrent placental dysfunction (Rasmussen, Irgens et al. 1999).

Thrombophilia is shown to be associated with placental abruption, severe preeclampsia, intrauterine growth restriction (IUGR), stillbirth and recurrent miscarriage. Established thrombophilic associations to placental abruption are protein S deficiency, APC resistance, factor V Leyden, hyperhomocysteinemia, factor IIG 20210A and antiphospholipid syndrome (Kupferminc 2003). In one study, 65% of women with placental abruption, severe preeclampsia, IUGR or stillbirth had inherited or acquired trhombophilia, compared to 18% in women with normal pregnancies (Kupferminc, Eldor et al. 1999). Several observational studies report folate deficiency and homocystein metabolic defects as risk factors for placental abruption, preeclampsia and spontaneous abortion. However, these changes may also just be markers for other risk factors associated with placental abruption (Ray and Laskin 1999).

The placental connective tissue might be more vulnerable in women predisposed for placental abruption. Vitamin C is required for collagen synthesis and maintenance of vascular structures (Barnes 1975). Reduced levels of ascorbic acid is reported in pregnancies complicated with preeclampsia and/or SGA-pregnancies, but there is inconsistency about the effect of vitamin C supplement (Chappell, Seed et al. 1999; Zhang, Williams et al. 2002; Beazley, Ahokas et al. 2005). An experiment with maternal vitamin C deficiency in swine has shown hemorrhages and haematomas in the placenta (Wegger and Palludan 1994). There is one report on reduced ascorbic acid in placental abruption, but without information about preeclampsia and SGA. Lowered ascorbic acid could just be a sign of a placental pathology rather than a cause (Sharma, Walzman et al. 1985). In addition, among women with placental abruption there are

also reports indicating altered immune response of the mother to the fetus (Matthiesen, Berg et al. 1995; Steinborn, Seidl et al. 2004).

Circulation

Mechanical properties of arteries

The arterial wall consists of three concentric layers, the tunica intima, tunica media and tunica adventitia. In the tunica intima there is an inner single cell endothelium layer and a thin basement membrane with elastin and collagen. The tunica media contains elastin, collagen, smooth muscle cells and non-fibrous matrix. With the distance from the heart the elastin-collagen ratio decrease and the arteries stiffen (Fischer and Llaurado 1966). Tunica media is the largest part of the arterial wall and its structure and thickness are the major determinants of the mechanical properties of the arteries. The adventitia, the outer layer, consists of fibroblasts and collagen fibres and is not considered to substantially contribute to the mechanical properties of the artery.

The most distensible segment of the arterial tree is the proximal aorta (Harkness, Harkness et al. 1957). The elasticity or distensibility of arteries is the capacity to deform under force (systole) and reform when the force is removed (diastole). This ability ensures a continuous perfusion of the body when the blood is partly stored in the arteries during systole and drained under diastole. The force/pressure per unit area of the vessel wall that causes the deformation /distension of the arterial wall is called stress. The ratio of the deformation to the original form of the arterial wall is called strain.

The deformation/distension of the vessel wall depends on the wall composition, the stress magnitude (blood pressure, tensile stress) and the rate (blood flow, shear stress) the stress is applied. Arterial tissue becomes stiffer when stretched, by for instance increased blood pressure. Arterial stiffness is inversely related to arterial distensibility, and arterial compliance is related to arterial distensibility and arterial volume. There is a high correlation between the mechanical function and the disposition of collagen and elastin in the arterial wall. The composition, architecture and thickness of the wall are determined by the stresses imposed by pressure and flow (Dobrin 1978; Glagov, Vito et al. 1992).

When blood pressure rises, the elastic fibres (mainly elastin) stretch and the vessels dilate and become stiffer. The collagen fibres are stiffer and offer counter-pressure after some dilatation. With increasing blood pressure collagen progressively replaces elastin and the stiffer collagen becomes load-bearing. The pressure-diameter relationship will be biphasic and the relation of stress to strain non-linear (Berry 1978; Dobrin 1978; Lanne, Stale et al. 1992).

Arterial remodelling

During human pregnancy there is a gradual remodelling of the fetal arterial walls where elastin and collagen increase differently. The rise of elastin is more rapid at the end of pregnancy and collagen increases more in the first period (Berry, Looker et al. 1972; Bendeck, Keeley et al. 1994).

The properties of the arterial wall change with ageing, when the elastin-collagen ratio decreases, but this change differs in different parts of the arterial tree (Dobrin 1978; Maurel, Shuttleworth et al. 1987). With age the arteries dilates and becomes functionally stiffer at physiological pressures. The abdominal aorta is more prone to degenerative changes than the common carotid artery (Dobrin 1978; Lanne, Hansen et al. 1994). Ageing and increased blood pressure are the major determinants of arterial stiffening. The effect of ageing seems to be a natural process and not simply a pathologic process of atherosclerosis (Kawasaki, Sasayama et al. 1987; Liao, Arnett et al. 1999; Cheng, Baker et al. 2002).

Regulation of blood pressure and cardiac load are influenced by the mechanical properties of large arteries. With increased blood pressure, adaptive changes of remodelling in the arterial walls are initiated. According to the Folkow hypothesis even mild stress, if sustained, may lead to a structurally amplified systemic resistance maintained also at vasodilatation (Folkow 1987). Then smooth muscle activations may lead to exaggerated resistance activation and this tends to accentuate the structural remodelling and may establish hypertension (Folkow 1987; Folkow and Svanborg 1993; Nichols WW 1998). Arterial stiffness is associated with cardiovascular disease and atherosclerosis, but the major determinants are age and hypertension. Diabetes and smoking seem to accelerate the stiffening (Levenson, Simon et al. 1987; Kool, Hoeks et al. 1993; Arnett, Evans et al. 1994; Hu, Wallensteen et al. 1996; van Popele, Grobbee et al. 2001).

Estimation of arterial stiffness and analyse of arterial pulse waveform

Arterial stiffness is a dynamic property influenced by all factors which indirectly or directly influence blood pressure. Measurements of elastic properties should be made under rest and standardised circumstances by a well-trained investigator (Van Bortel, Duprez et al. 2002). After recommendations from the First International Consensus Conference on the Clincal Applications of Arterial Stiffness the trend is to use compliance coefficient (CC), distensibility coefficient (DC) and pulse wave velocity (PWV) (Van Bortel, Duprez et al. 2002). PWV measurements are suitable for research in fetuses as blood pressure measurements, which are impossible to obtain in the fetus, are not necessary. PWV is mainly determined by two major factors: the blood pressure and the stiffness of the vessel wall (Lindstrom K, Gennser G et al. 1987; Blacher, Asmar et al. 1999; Cheng, Baker et al. 2002). The higher the blood pressure, and/or the lower the stretching capacity of the vessels, the more dynamic is the pulse wave, resulting in an increased PWV.

Analysis of the diameter changes and the arterial pulse wave form is a complementary way of obtaining information about the mechanical properties of the arteries. Strain can be estimated through the arterial diameter in systole and diastole, and maximum incremental velocity (MIV), the dilatation speed, through the first derivative of the diameter change in systole (se fig 1 under methods). There is a positive correlation of MIV to cardiac contractility and a negative correlation to total peripheral resistance (Gustafsson, Stale et al. 1989).

<u>Definition of vessel stiffness parameters (Van Bortel, Duprez et al. 2002)</u>

Compliance coefficient (CC) is the change of cross-sectional area (A) per unit of pressure (P).
$CC = \Delta A/\Delta P$.
Distensibility coefficient (DC) is defined as the relative change in cross-sectional area.
$DC = (\Delta A/A)/\Delta P$.
Pulse wave velocity (PWV) measures the distance the pulse wave travels in a certain time and is inversely related to DC ($PWV = \sqrt{1/\rho * DC}$, where ρ is the blood density).

<u>Other parameters for estimation of mechanical properties in arteries</u>
Minimum diameter in diastole (Dd)
Maximum diameter in systole (Ds)
Maximum incremental velocity (MIV), the dilatation speed during systole, defined as the maximum first derivative of pulse wave upstroke
Pulse amplitude (ΔD), the difference between maximum diameter in systole (Ds) and minimum diameter in diastole (Dd).
Strain (ΔD /Dd), the ratio of the deformation to the original form

Fetal circulation

The fetal circulation also includes the placenta where the oxygenation takes place i.e., the feto-placental unit. In the central fetal circulation, the ductus arteriosus and the foramen ovale are responsible for the specific fetal orientation of the blood streams, for loading of oxygen in the placenta and for achievement of appropriate oxygenation. In normal pregnancy the placenta is a low resistance organ. In compromised pregnancy vascular pathology often occur which may result in increased peripheral resistance that might impair fetal circulation. Placental vascular pathology may be seen in preeclampsia, intrauterine growth retardation, placental abruption, preterm birth, diabetes and smoking (Naeye, Harkness et al. 1977; Asmussen 1980; Khong, De Wolf et al. 1986; Salafia, Vogel et al. 1991; Laurini, Laurin et al. 1994; Saldeen, Olofsson et al. 2002). The utero-placental circulation is in clinical practice commonly monitored by Doppler ultrasound in the umbilical cord and the uterine arteries.

AIMS OF THE STUDY

1) To study maternal smoking in relation to preterm birth
    a) Is smoking a risk factor for preterm birth?
    b) What is the relation between smoking and onset of labour in preterm birth?
    c) What is the relation between smoking and gestational age in preterm birth?
    d) What is the relation between smoking and different causes of very preterm birth?

2) To study the relation of maternal smoking to placental abruption
    a) To estimate the risk of smoking-related risk of placental abruption
    b) Is smoking a risk factor for perinatal death in pregnancies without placental abruption?
    c) What is the relation of smoking to perinatal mortality in pregnancies with placental abruption

3) To study the relation of daily maternal smoking to pulse wave characteristics in the fetal aorta.

MATERIALS AND METHODS

Setting and Design

All studies were performed in Sweden. Studies presented in papers I, II and III were retrospective, but exposure and outcome information was based on prospectively registered information from standardized antenatal, obstetrical, and neonatal records. Papers I and III present large cohort studies, where data were retrieved from population-based registers. Paper II presents a case-control study, performed on 295 cases and 295 controls. Information for this study was collected from patient records at Danderyd Hospital and South General Hospital (Södersjukhuset) in Stockholm. Paper IV is a prospective clinical study including 34 smokers and 34 non-smokers enrolled from antenatal clinics in Stockholm. Data for the analyses were obtained by means of an ultrasonic examination of the fetal aorta.

Data sources (Papers I, II and III)

Registers (Papers I and III)

Papers I and III are based on data retrieved from the large nation-wide Swedish Medical Birth Register (MBR), held by the Swedish National Board of Health and Welfare. This register includes at least 98% of all births in Sweden. The register was established in 1973 after the introduction of a standardized set of medical records used by all antenatal care clinics and delivery units. Copies of these records are sent to the Swedish National Board of Health and Welfare, where the information is computerized (Cnattingius, Ericson et al. 1990; SoS 2002).
Starting at the first visit to the antenatal clinic, data is prospectively collected by midwifes who interview and examine their patients. More than 95% of all women attend antenatal care in Sweden before 15th week of pregnancy (Lindmark and Cnattingius 1991). At the first antenatal visit women provide information about previous reproductive history, smoking habits, state of health, weight, height and family situation. At delivery, notes are taken about maternal age, time of labour start, time of rupture of membranes, onset of delivery (spontaneous, vaginally induced, or Caesarean section before onset of labour), mode of delivery (vaginal, vaginal instrumental, or caesarean delivery), time of birth, gestational age, infant's sex, birth weight, birth length, and head circumference, whether it was a live or a stillbirth, a single or a multiple birth and information on Apgar scores. At discharge of mother and infant from the hospital, the gynaecologist codes complications and diagnoses during pregnancy and delivery, and the paediatrician codes the neonatal diagnoses according to the Swedish version of the International Classification of Diseases (ICD).

Births and deaths are validated each year by linking this register to the Register of Total Population and Population Changes, held by Statistics Sweden (SCB) (SoS 2002). This is possible through the unique personal identification number that is assigned to each inhabitant in Sweden. Data about length of the mother's formal education in years is obtained from the Education Register, which may be linked to the MBR. This register includes information about length of highest completed level of formal education, from elementary to postgraduate level (SCB 1996).

Definition of data and diagnoses used in register studies (Paper I and III)

The following information was derived from the MBR: data on maternal age at delivery, parity (including stillborn), years of infertility, smoking habits (daily smoking or not), years of formal

education, cohabitation with infant's father, gestational age, birth weight, spontaneous or induced onset of delivery. SGA was defined as a birth weight less than two standard deviations below the mean estimated fetal weight for gestational age according to the Swedish standard curve (Marsal, Persson et al. 1996). The following diagnoses and ICD-9 codes were used: placental abruption (641C), placenta praevia (641 A and 641B), essential hypertension (642A), gestational hypertension (642D), mild preeclampsia (642E), severe pre-eclampsia (642F), diabetes (250), gestational diabetes (648W), congenital anomalies (740-759), congenital heart anomalies (745-747), and patent ductus arteriosus (747A). We also used information on PROM (658B) and preterm PROM (code 658B combined with gestational age less than 37 completed gestational weeks).

Patient records (Paper II)

Delivery wards in Sweden hold "a ledger" ("förlossningsliggare" in Swedish), over all registered births. For Paper II, the delivery ledgers and individual records for two hospitals in Stockholm (Danderyd Hospital and Stockholm South General Hospital) were scrutinised by one of the authors (N.K-B), with the ambition to find all cases of very preterm singleton live births during a certain period. These ledgers include information on time of birth, birth weight, gender, presentation (head, breech), single or multiple birth, if live or stillbirth and generally also provide information on gestational age and/or estimated time of birth. From these ledgers, it is possible to obtain the unique personal number of deliveries of interest, and through this order the individual antenatal, obstetrical, and neonatal record stored at the hospital archives. Very preterm birth was defined as a gestational age of $\leq$32 weeks and 0 days. When gestational age and estimated time of birth was missing in the ledgers, all individual records for those with a birth weight less than 2500 g were checked. To every very preterm birth that was found, a live singleton term birth registered immediately after the very preterm birth was chosen to be included in the control group. If the personal record was not found the next term pregnancy registered was chosen. Obstetrical records could not be traced in six cases of the preterm births that were identified in the ledgers. Of the 295 very preterm birth that were included in the study, 144 were delivered at Danderyd Hospital and 151 at South General Hospital in Stockholm.

Data was abstracted following a strict protocol (Table 2). Causes of delivery were classified hierarchically: labour contractions, rupture of membranes, pregnancy bleedings, hypertensive diseases, intrauterine growth restriction (IUGR) and other causes. All registered data and diagnoses were checked for accuracy in the written reports.

Table 2. Extraction form for retrieval of data for Paper II. Definitions added**.**

Data	Definitions
Maternal characteristics	
Age	Years at delivery
Weight	Prepregnancy weight (kg)
Length	Cm
Cohabitation	With infants father, yes or no
Profession	Coded to classify socio-economic status (SES) according to the recommendations set forth by Statistics, Sweden (SCB) (SCB 1995).
Smoking habits	No smoking, moderate (1-9 cigarrettes per day) and heavy smoking (≥10 cigarrettes per day) at registration to antenatal care
Previous reproductive history	
Previous spontaneous abortion	Number
Previous induced abortion	Number
Parity	Previous births including stillbirths plus the current birth
Previous preterm births	Including stillbirths.
Causes of preterm birth (Spontaneous and induced)	
Preterm labour	Onset of labour before or within 24 hours after the rupture of membranes
Preterm PROM	Rupture of membranes at least 24 hours before onset of labour
Late pregnancy bleedings	Placental abruptio and placenta praevia (classification according to ICD)
Hypertensive diseases	Hypertension, gestational hypertension and preeclampsia (classification according to ICD)
IUGR	More than two standard deviations below the estimated size for gestational age at ultrasonic examination of the fetus in utero
Other causes	Asfyxia of unknown cause, emergency abdominal surgery, cancer, status epilepticus, severe diabetes, iatrogenic due to medical interventions.
Risk factors for preterm labour	
Infection	Temperature >38° C and/or elevated C-reactive protein >50 mg/l at admission to hospital or developing during delivery or obvious gastroenteritis
Hydramniosis	
Major anomaly of uterus	Amniotic banding, uterine duplicity, total atresia of one half of the uterus, total septum
Major fetal anomaly	Atresia of intestines, terisomi 18, diabetic anomalies
Previous conisation of cervix	All kinds (cold knife, cryo surgery, laser, loop diathermy)
Infant characteristics	
Gestational age	Weeks and days
Birth weight	Grams

Ultrasound study (Paper IV)

Participants (Paper IV)

Thirty-four women who were daily smokers, with a pregnancy of 31 to 40 gestational weeks, were enrolled. To every smoker a non-smoker matched for maternal age and gestational week was recruited. In the matched pairs no difference of more than seven days in gestational age and five years in maternal age was accepted. Informed consent was obtained from all participants. The participants had to be healthy without chronic diseases or pregnancy complications. Women who developed pregnancy complications after examination were excluded.

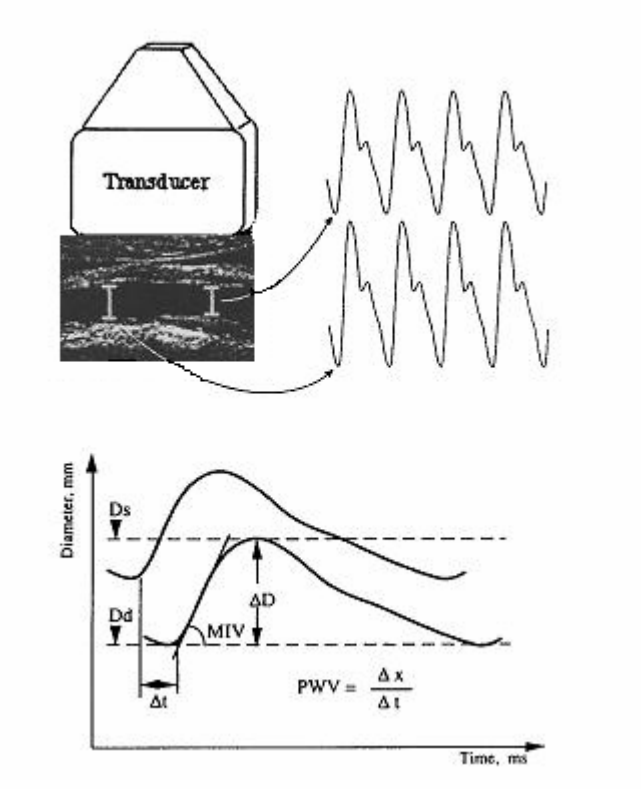
To be accepted for examination, smoking participants should not have smoked for at least 10 hours before the investigation. On arrival all participants were checked for carbon monoxide (CO) in the expiratory air, and for the presence of serum cotinine, a metabolite of nicotine. To avoid exclusion the value of CO had to be <10 ppm. Cotinine is eliminated from the body much more slowly than nicotine, and is widely used for validation of smoking habit (Bardy, Seppala et al. 1993; Lambers and Clark 1996). We used cotinine in serum to verify self-reported exposure status, with >15 ng/ml as the cut-off limit for active smoking.

Ultrasonic measurements (Paper IV)

The mechanical properties of the fetal aorta were assessed by means of an ultrasonic system, comprising a real-time scanner (Hitachi EUB-240, Tokyo Japan) fitted with a
5 MHz linear array transducer, interfaced with two pairs of custom-built zero-crossing phase-locking echo-trackers (Diamove®, Lund, Sweden) and a type 486 desk top computer including a hard disc and a mathematic co-processor.

In this technique the sonic B-mode image of a straight segment of the fetal thoracic aorta between its arch and the diaphragm is chosen and two pairs of markers, representing the echo-trackers, are positioned into the image of the aortic lumen. The trackers, with a phase-locked loop circuit controlling an electronic gate, automatically lock to the echoes from the anterior and posterior walls, respectively, and the aortic diameter with its changes is measured continuously in a plane, perpendicular to the longitudinal axis of the vessel. (Se Figure 1) The radio-frequency ultrasonic signals are sampled at a frequency of 100 MHz, implying that the system can detect shifts in target distance down to 7.8μm, and the repetition frequency of the phase locked loop circuits is 870 Hz, which gives a time resolution of approximately 1.2 msec (Lindstrom K, Gennser G et al. 1987; Benthin, Dahl et al. 1991).

Figure 1. Illustration of the ultrasonic echo-tracking system monitoring the vessel wall pulsation in two positions. The vessel diameter, pulse amplitude, and maximum incremental velocity are obtained from the pulse wave forms; the pulse wave velocity is calculated from the small time shift between the two curves (Retrieved with permission from Dr Jie Hu, Thesis Karolinska Institutet, Stockholm 1998)



Definition of outcome measures (Paper IV)

The recordings should have a sequence of at least six consecutive cycles to be accepted for calculation. It is desirable to have at least three acceptable recordings for calculation of a mean value to represent each parameter. The pulse waveform variables and the pulse wave velocity were calculated by a software program (Lindstrom K, Gennser G et al. 1987). Values computed were (abbreviation of variable and units in brackets):

- End-diastolic diameter of the artery (Dd; mm), the minimum diameter in diastole
- Peak systolic diameter of the artery (Ds; mm), the maximum diameter in systole
- Maximum incremental velocity (MIV; mm/sec), the maximum first derivative of pulse wave upstroke or the systolic dilatation speed
- Fetal heart rate (beats per min)
- Pulse wave velocity (PWV; m/sec), the distance between the two recording sites divided by the transit time for the pulse wave.

Calculations from the primarily measured variables were made for:

- Pulse amplitude ($\Delta D = Ds - Dd$; mm)
- Strain ($\Delta D / Dd$), a way of estimating vessel stiffness

Presentation of papers

An overview of all papers on subjects, design, outcome measures and covariates is presented in Table 3.

Paper I

To study the effects of maternal smoking as a risk factor on spontaneous and induced preterm birth in relation to severity of preterm birth, a population-based cohort study based on the MBR was performed. From 1991 to 1993, we identified 352 609 live singleton births. To increase the homogeneity of the study population, we excluded women born outside Nordic countries (n=40 211) and 421 pregnancies where information on gestational age was missing. The remaining 311 977 births constitute the study population.

Preterm birth (<37 weeks) was stratified into very preterm birth (less than 32 weeks) and moderately preterm birth (33-36 weeks). In the analyses of smoking and risk of preterm birth, adjustment was made for confounding factors as maternal age, parity and education. To investigate whether smoking-related risks of preterm birth was mediated by smoking-related pregnancy complications, pregnancies complicated with placental abruption, placenta praevia, preterm PROM, IUGR and hypertensive diseases were excluded in one model of risk estimation. To further clarify the effect of maternal smoking on very and moderately preterm birth, stratification was made by onset of delivery (spontaneous and induced onset).

Paper II

To investigate the association between smoking and causes of very preterm birth, a case-control study was conducted on data obtained from individual patient records, using a standardized extraction form. From 1988 to1992 the hospital local delivery ledgers in two large hospitals in Stockholm (Danderyd Hospital and South General Hospital) were examined to find all live singleton very preterm births (defined as a gestational age from 22 gestational weeks and 0 days to 32 weeks and 0 days). Records for 295 very preterm births were found. To every very preterm birth, a live singleton term birth (gestational age ≥37 weeks) registered immediately after the case was chosen as control.

Causes of very preterm birth were hierarchically classified as preterm labour, preterm PROM, pregnancy bleeding (placental abruption and placenta praevia), hypertensive diseases (essential and gestational hypertension and preeclampsia), IUGR and other causes. Some cases with preterm labour also had disorders known as risk factors for preterm birth, such as infections, previous conisation of the cervix, major fetal or uterine anomalies and hydramniosis. After exclusion of such cases, we defined a group, called ‘idiopathic’ labour. Smoking-related risks of very preterm birth by cause were calculated, before and after adjusting for possible confounders (maternal age, parity, previous preterm birth, induced and spontaneous abortions, socio-economic status [SES], body mass index [BMI] and height).

Paper III

To study smoking as a risk factor for perinatal death in cases of placental abruption a population-based cohort study was conducted. From 1987 to 93, we identified 795 459 singleton births with complete information about gestational age from the MBR.

To begin with, risk factors, confounders, maternal pregnancy complications and birth outcomes related to risk of placental abruption were presented. Analysed possible confounders included maternal age, parity, cohabitation, education and infertility. Other analysed covariates were

hypertensive diseases, diabetes, preterm PROM, congenital anomalies, SGA and gestational age. We estimated smoking-related risks of placental abruption, as well as smoking-related risks of perinatal death in cases with and without placental abruption. To further clarify the role of smoking as a risk factor of perinatal death in cases of placental abruption, the analyses were stratified into term and preterm birth.

Paper IV

To achieve information on whether chronic maternal smoking might have any detectable impact on the mechanical properties of fetal aorta, pulse waves in the fetal aorta were analysed. The participants were 34 smoking and 34 non-smoking healthy women with singleton pregnancies, in week 31 to 40. To every smoker, a non-smoking control matched for maternal age and gestational week was recruited. Results were computed on 32 smokers and 30 non-smokers. Four of the non-smokers and two of the smokers were excluded due to later developing pregnancy complications or failure in following the protocol (preeclampsia, delivery of a growth-retarded baby). There were12 moderate smokers and 20 heavy smokers. Each participant underwent ultrasonic investigation once in pregnancy. From the 31st to the 36th gestational week 15 smokers and 16 non-smokers were examined, and the rest from 36th week to the 40th week. Data on PWV, Dd, Ds, and MIV in the fetal aorta were obtained by means of ultrasonic computerized equipment. From the primarily measured variables the pulse amplitude ΔD (Ds - Dd) was estimated as well as strain (ΔD/Dd). Data of each parameter was related to gestational age to reveal the trend over time, and the trends for smokers and non-smokers were compared.

Table 3. Overview of papers.

Paper	Subjects	Design	Outcome measures	Covariates
I	Single births in Sweden to women born in Nordic Countries 1991-1993 n=311 977	Population-based cohort study with data from the Medical Birth Register	Preterm birth, stratified in: very and moderately preterm birth spontaneous and induced delivery	Maternal smoking Maternal age, parity Education, placental abruption, placenta praevia, preterm PROM, IUGR, hypertensive diseases
II	Single births in Stockholm 1988-1992 295 cases and 295 controls	Case-control study based on data from personal records	Very preterm birth	Maternal smoking Causes of very preterm birth: preterm labour, 'idiopathic' preterm labour, preterm PROM, pregnancy bleedings, hypertensive disease, and other causes.
III	Single births in Sweden 1987-1993 n=795 459	Population-based cohort study with data from the Medical Birth Register	Placental abruption, Perinatal death Perinatal death in cases of AP	Maternal smoking Maternal age, parity, cohabitation, education, infertility, preterm PROM, hypertensive diseases, diabetes, congenital anomalies, congenital heart anomalies, SGA, gestational age
IV	Single births in Stockholm 34 smokers 34 non-smokers	Prospective clinical study based on data from ultrasonic investigation of the fetal aorta. Matching was made for gestational and maternal age.	PWV Dd ΔD MIV Strain	Gestational age

Statistical Methods

Papers I, II and III

Logistic regression analyses were used to estimate the effect of maternal smoking on risk of preterm birth (Paper I and II), on placental abruption (Paper III), and on perinatal death in pregnancies complicated with placental abruption (Paper III). In Paper I, logistic regression analyses were used to study the relation of smoking to very and moderately preterm birth, before and after stratification for onset of delivery. In Paper II, it was used for estimating the effect of smoking on very preterm birth by cause. In Paper III, we used logistic regression analyses to estimate the effect of smoking on placental abruption and perinatal death. To measure the independent effect of smoking, analyses were adjusted for possible confounders. Odds ratios (ORs) were used to estimate relative risks, using 95% confidence intervals (CI).

Smoking-related pregnancy complications might be intermediate variables for the effect of smoking on preterm birth. In the study of preterm birth (Paper I), we therefore excluded pregnancies with placental abruption, placenta praevia, preterm PROM, IUGR, and hypertensive diseases in an additional model. Similarly when we studied smoking-related pregnancy complications and risk of placental abruption (Paper III), we only presented in crude ORs. All the statistical analyses were performed with the logistic procedure in the SAS programme package (SAS 1993).

Paper IV

Correlation analysis by linear regression was used to analyse the relation of PWV, Dd, ΔD , strain and MIV to gestational age. For comparison of changes in pulse wave variables in smokers and non-smokers, the interaction effect of smoking habit with gestational age was estimated in a multiple linear regression model. A p-value less than 0.05 was regarded as statistically significant. The statistic analyses were performed with Statview programme, version 4,5.

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RESULTS

Descriptive data in Papers I and II

In Paper I, there were in all 311 977 births, of which 1% were very preterm births (≤32weeks) and 4.2% were moderately preterm birth (33 – 36 weeks). Information about onset of delivery was missing in 7.1% of the study population (17.2% in the very preterm births and 10.0% in the moderately preterm births). For preterm birth with known onset of delivery, spontaneous onset was more common than induced onset: 64% of very preterm births and 76% of moderately preterm births. Rates of preterm birth varied with maternal age and parity: lowest rates were found among mothers aged 25 to 29 years, and in women with parity 2 to 3. The rate of preterm birth decreased with increasing level of education (Paper I).

The case-control study, was nested in a cohort of 41 741 live births. The over all rate of very preterm birth in this cohort was 0.72% (Paper II). In the case-control study, missing information on profession, parity and previous abortions varied from 0.5% to 5%. For BMI and height missing values were higher: in very preterm birth (cases), 15.6% and 11.2%, respectively. In the term births (controls) corresponding rates were 6.4% and 4.4%, respectively.

Maternal smoking and pregnancy complications (Papers I and II)

Maternal smoking was associated with dose-dependent increases in rates of placental abruption, placenta praevia, preterm PROM and IUGR and decreasing rates of hypertensive diseases (Paper I). In Paper II smoking was analysed in relation to causes of very preterm birth (Table 4). Cases classified as IUGR and "other causes" were not analysed due to small numbers. Smoking seems to be a risk factor for very preterm birth caused by preterm PROM and a strong risk factor for pregnancy bleedings, but not for hypertensive diseases (Paper II).

Table 4. Risk of very preterm birth by pregnancy complications according to maternal daily smoking. Adjusted* odds ratios (OR) and 95% confidence intervals (CI). Paper II.

	Preterm PROM	Pregnancy bleedings[†]	Hypertensive diseases[‡]
Smoking habit	Adjusted* OR (95% CI)	Adjusted* OR (95% CI)	Adjusted* OR (95% CI)
Not smoking[§]	1.0	1.0	1.0
1-9 cig/day	1.5 (0.6-3.8)	1.4 (0.5-4.2)	0.5 (0.2-1.5)
≥10 cig/day	2.6 (0.9-7.6)	4.7 (1.6-14.2)	1.1 (0.3-3.6)

* Adjusted for maternal age, parity, previous preterm birth, induced and spontaneous abortions, SES, BMI, height.

[†] Placental abruption and placenta praevia

[‡] Essential hypertension, pregnancy hypertension, preeclampsia

[§] Reference group

Maternal smoking and preterm birth (Papers I and II)

Information about smoking habit was missing in 4.3% of the cohort of 311 977 births, and among these the rate of preterm birth was 9% (Paper I). In the case-control study, information about smoking habit was missing in 4% of the 295 cases of very preterm birth and in 5% of the 295 controls (Paper II). In the cohort of 311 977 births (Paper I), the risks of very and moderately preterm birth increased with amount smoked. Excluding pregnancies with smoking-related pregnancy complications did not essentially change these risks (Table 5).

Table 5. Adjusted odds ratios (OR) and 95% confidence intervals (CI) of preterm birth by maternal smoking habits. N=311 977 births. Paper I.

	≤32 weeks				33 - 36 weeks			
	Model 1*		Model 2†		Model 1*		Model 2†	
Maternal smoking	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)
Not smoking‡	1.0		1.0		1.0		1.0	
1-9 cig/day	1.3	(1.2-1.5)	1.2	(1.1-1.5)	1.1	(1.1-1.2)	1.2	(1.1-1.2)
>10 cig/day	1.6	(1.4-1.8)	1.6	(1.3-1.9)	1.4	(1.3-1.4)	1.4	(1.3-1.5)

* Adjusted for maternal age, parity and education

† Adjusted for maternal age, parity and education and excluding pregnancies with pregnancy complications (placental abruption, placenta praevia, preterm PROM, IUGR and hypertensive diseases

‡ Reference group

In the large cohort (Paper I) we were also able to demonstrate that smoking had a stronger influence on spontaneous than on induced preterm birth, especially in very preterm birth (Table 6).

Table 6. Adjusted* odds ratios (OR) and 95% confidence intervals (CI) for spontaneous and induced preterm birth by maternal smoking habits. N=311 977 births. Paper I.

	≤32 weeks				33 - 36 weeks			
	Spontaneous delivery		Induced delivery		Spontaneous delivery		Induced delivery	
Maternal smoking	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)	OR	(95%CI)
Not smoking†	1.0		1.0		1.0		1.0	
1-9 cig/day	1.4	(1.2-1.6)	1.2	(1.02-1.5)	1.2	(1.1-1.2)	1.0	(0.8-1.1)
>10 cig/day	1.7	(1.4-2.0)	1.3	(1.1-1.6)	1.4	(1.3-1.5)	1.2	(1.03-1.3)

* Adjusted for maternal age parity and education

† Reference group

Maternal smoking was also associated with dose-dependent increases in risks of very preterm birth in the small cohort (Paper II). Compared with non-smokers, adjusted OR:s (95% CI) of very preterm birth were1.4 (0.8-2.4) among moderate smokers and 2.9 (1.5-5.7.) and among heavy smokers.

To clarify the effect of smoking on very preterm birth, smoking habits were analysed in relation to causes of very preterm birth (Paper II). Causes were preterm labour (37.3%), preterm PROM (23.7%), late pregnancy bleedings (13.9%), constituted of 37 pregnancies with placental abruption and four with placenta praevia), hypertensive diseases (20.7%), IUGR (2%) and other causes (2.4%). Within the group of very preterm birth caused by preterm labour, we also defined a subgroup called 'idiopathic' preterm labour. In this group pregnancies with known determinants for preterm labour were excluded (infections, conisation of cervix, hydramniosis, and major anomalies of the uterus or the fetus). Logistic regression analyses showed that maternal smoking was a risk factor for very preterm birth caused by preterm labour, and probably also for 'idiopathic' preterm labour (Table 7).

Table 7. Risk of very preterm birth by preterm labour and 'idiopathic' preterm labour according to maternal daily smoking. Adjusted* odds ratios (OR) and 95% confidence intervals (CI). Paper II.

	Preterm labour		'idiopathic' preterm labour†	
Smoking habit	Adjusted* OR (95%CI)		Adjusted* OR (95%CI)	
Not smoking‡	1.0		1.0	
1-9 cig/day	1.9	(1.0-3.6)	2.4	(1.2-5.0)
≥10 cig/day	2.6	(1.1-6.1)	2.2	(0.9-5.7)

* Adjusted for maternal age, parity, previous preterm birth, induced and spontaneous abortions, SES, BMI, height

† Cases of preterm labour after exclusion of cases with infection, conisation of cervix, hydramniosis, major uterine and fetal anomalies.

‡ Reference group

Descriptive data in Paper III

In 795 459 singleton pregnancies, there were 4003 placental abruptions (0.5%).
Information about smoking was missing in 6.4% in the entire cohort, while among those having placental abruption, information was missing in 9%. In cases of perinatal deaths in pregnancies with placental abruption, loss of information on smoking habit was 15.1%.

Risk factors of placental abruption (Paper III)

Significant risk factors for placental abruption were maternal age ≥30 years, parity 3 or more, no cohabitation with infant's father, low level of formal education, smoking, and infertility. The following pregnancy complications were also associated with increased risks of placental abruption: hypertensive diseases, pregestational diabetes, preterm PROM, SGA, low gestational age and congenital heart anomalies.

Perinatal death in pregnancies without placental abruption (Paper III)

In pregnancies without placental abruption (n=791 456), there were 4111 perinatal deaths (0.52%). In pregnancies without placental abruption, the following factors were associated with increased risks of perinatal mortality in the adjusted analysis: heavy smoking, low level of education, maternal age ≥30 years (especially ≥35 years), and years of infertility. In addition, essential hypertension, severe preeclampsia and pregestational diabetes also increased the risk.

Perinatal death in pregnancies with placental abruption (Paper III)

In pregnancies with placental abruption there were 424 perinatal deaths (10.6%). In pregnancies with placental abruption, smoking and severe preeclampsia increased the risk of perinatal death. In pregnancies with placental abruption, we found that heavy smoking increased the risks of perinatal mortality in both preterm and term birth (Table 8).

Table 8. Perinatal death in 4003 pregnancies with placental abruption.
Crude odds ratios (OR), 95% confidence intervals (CI). Paper III.

	<37 weeks n=2231		≥37 weeks n=1772	
	Perinatal deaths	Crude OR (95% CI)	Perinatal deaths	Crude OR (95% CI)
Not smoking*	127	1.0	47	1.0
1-9 cig/day	72	1.3 (0.9-1.8)	25	1.7 (1.0-2.8)
≥10 cig/day	67	1.6 (1.1-2.2)	22	2.1 (1.2-3.5)
Severe preeclampsia				
No*	294	1.0	104	1.0
Yes	21	1.6 (1.0-2.7)	5	2.5 (0.9-6.4)
SGA				
No*	245	1.0	93	1.0
Yes	65	1.9 (1.4-2.6)	10	1.2 (0.6-2.3)

* Reference group

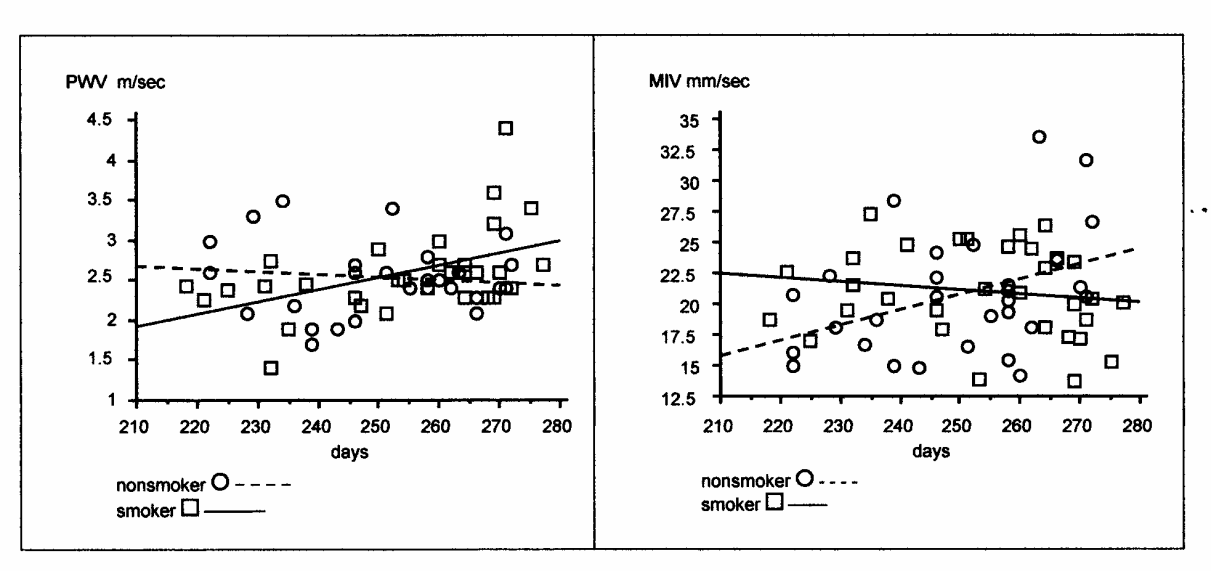
Descriptive data in Paper IV

A non-significant difference of 197 g in birth weight was found between infants of non-smokers and smokers. Among the non-smokers there were no detectable levels of serum cotinine except in two cases where there were traces (0.9 and 1.2 ng/ml, respectively), whereas levels exceeding 5 ng/ml were found in all smokers. The serum cotinine level in heavy smokers was significantly higher ($p<0.02$) compared to moderate smokers. Those with a serum cotinine level >90ng/ml all smoked more than 10 cigarettes daily and all of gave birth to infants weighing less than expected according to gestational age. Between the non-smokers and smokers there was no difference in maternal heart rate, maternal systolic and diastolic blood pressure.

Maternal smoking and pulse wave measurements in the fetal aorta (Paper IV)

Linear regression analyses of the relation of fetal aortic pulse wave parameters to gestational age were made. PWV increased with gestational age only among the smokers ($p<0.006$), and remained unchanged among the non-smokers. For PWV a difference between the regression slopes of smokers and non-smokers was seen (p-value for interaction <0.02). During gestation MIV remained unchanged in the smokers, and increased among the non-smokers ($p<0.002$), and also in this case there was a difference between the regression lines ($p<0.02$). Regression lines of PWV and MIV are presented in Figure 2.

Figure 2. Linear regression. PWV and MIV to gestational age. Non-smokers and smokers. Paper IV.



Dd increased with gestational age in a similar way in both groups. Strain decreased and no difference was found between the groups, although the decrease was significant only among smokers ($p<0,005$). There was no significant change of fetal heart rate (FHR) with gestational age. Dependence of the pulse wave parameters on estimated fetal weight (EFW) at the time of investigation was also checked, but only Dd was significantly dependent of EFW. All pulse wave parameters were independent of maternal age.

Loss of information in pulse wave measurements in the fetal aorta (Paper IV)

For computing the results we initially aimed at a mean value from at least three acceptable recordings for calculation of each parameter, but obtained curves with signs of fetal breathing and movements were expelled before calculation. Curves acceptable for calculation of data for statistical analysis are shown in Table 9. This shows that in 12% (four cases) the value for PWV was calculated from just one curve sequence, but if these cases were omitted from the analysis, PWV still increased by gestational age ($p = 0.003$).

Table 9. Pulse wave parameters. Obtained curves acceptable for calculating statistics in each object. In each curve at least six pulse waves were recorded. Paper IV

	Nonsmokers n=30		**Smokers** n=32	
	Acceptable curves			
(n)	PWV %	Dd,ΔD,MIV %	PWV %	Dd,ΔD,MIV %
0	0	0	3	0
1	3	0	12	0
2	0	0	18	12
3 or more	97	100	67	88

Summary of results

Maternal smoking is a risk factor for preterm birth, especially very preterm birth and spontaneous preterm birth. (Papers I and II)

Maternal smoking is a risk factor for preterm labour as a cause of very preterm birth. Maternal smoking probably also increases the risk for 'idiopathic' preterm labour. (Paper II)

Maternal smoking was also shown to increase the risk of very preterm birth caused by pregnancy bleedings, and probably also very preterm birth caused by preterm PROM.
(Paper II)

Maternal smoking is a risk factor for placental abruption. (Paper III)

Maternal smoking is a risk factor for perinatal deaths in births without and
with placental abruption. The smoking-related increased risk of perinatal death in pregnancies with placental abruption was found in both preterm and term births. (Paper III)

In fetuses of daily smoking mothers, the relation of PWV and MIV in the fetal aorta to gestational age seems to differ from that in fetuses with non-smoking mothers. (Paper IV).

GENERAL DISCUSSION

Methodological considerations

Internal validity

Definition of internal validity

Internal validity is the power of a finding or a test to measure what it is said to measure. The internal validity is high if systematic errors, confounding and the role of chance are at minimum (Hennekens and Buring 1987; Beaglehole R 1995).

There are two types of systematic errors: selection bias and observational bias.
Selection bias can occur whenever the inclusion of cases and controls into the study depends in some way on the exposure of interest. Selection bias is a particular problem in case-control studies, since exposure and disease have both occurred when subjects are selected for the study. Observational bias can be defined as errors in obtaining information from subjects once they are in the study. There are different types of observational bias: interviewer bias, misclassification bias, follow-up bias and recall bias.

Interviewer bias may occur when the interviewer or data abstractor is aware of the study hypothesis and/or of case – control status. This may be a concern in Paper II, when one of the authors (NK-B) extracted data from patient records. Misclassification bias of exposure and outcome may be a concern in Paper I-IV. Recall and follow-up bias are of no concern in the present studies.

Confounding, or confusion of effects, *can occur* if a factor is associated to both the exposure and the disease. A confounder has an independent effect on the disease and is unevenly distributed among the diseased. Confounding *exists* if you account for a covariate in the analyses, and thereby influence the risk related to the exposure of interest. Residual confounding is the possibility of confounding due to unmeasured factors, and should always be considered in observational studies. A confounder should be separated from an intermediate link in the causal chain between exposure and disease. For example, smoking is causally associated with fetal growth restriction, which in turn is causally associated with stillbirth. Thus, with regard to the association between smoking and stillbirth, fetal growth restriction is an intermediate link in the causal chain rather than a confounder.

Random error, or the role of chance, is the error that remains after systematic errors and confounding has been eliminated. There is always the possibility that the result of a study is due to chance, as most studies are made on samples from a larger population. With a larger study population, the role of chance decreases.

Medical birth registers

The MBR covers at least 98% of all births in Sweden. In registers of this size there are always errors: there are missing data in basic medical records, mistakes in the transferral of information to the National Board of Health, and in data entry into computer medium. Transferral data might be indistinct, not only because of bad handwriting and incomplete information, but the form may also be insufficient. In the data entry to MBR there is generally about 2% incorrectly transferred information. Varying and inexact diagnostic criteria in clinical practice may affect the validity of data in the MBR. There might be differences in diagnostic

habits and trends between hospitals, counties and over time, which can be seen in the distribution of ICD-codes. And ICD-codes may also be incorrect or missing (Cnattingius, Ericson et al. 1990; SoS 2002). In the last quality study of MBR 3.4% of the ICD-codes were found to be incorrect or that a better one could have replaced them, and that missing codes of not serious diagnoses were common. It seems to be a fairly good accuracy for placental abruption, preclampsia and gestational age, but not as good for PROM, IUGR and asfyxia for example (Cnattingius, Ericson et al. 1990; SoS 2002). Generally the statistical power in a study of a large nation-wide population compensates for such operator errors of the register. The fact, that data are collected prospectively and independently of a certain aim preclude bias.

In Paper I and III, we used MBR to perform large cohort studies. This large register made it possible to study a questioned risk factor associated with a moderate increase in relative risk, such as maternal smoking in preterm birth (Paper I). Moreover, we were also able to define a rare cohort of pregnancies with placental abruption (0.5% of all pregnancies), and to study risk factors for perinatal death within this cohort (Paper III). Thus, the large population-based cohort of pregnancies included in the MBR, makes it possible to study the effect of one exposure on several outcomes (maternal smoking and risks of preterm birth and placental abruption, and maternal smoking and risks of perinatal death in pregnancies with placental abruption). In addition it admits a study of an exposure together with other risk factors included in the MBR, followed by a control for these possible confounding factors. The large size also permits stratification of the cohort and exclusion of subgroups to clarify the effect of the exposure on outcome risks (Hennekens and Buring 1987; Beaglehole R 1995).

In Paper I, we wanted to investigate whether the smoking-related effect on preterm birth was mediated by smoking-related pregnancy complications. We therefore repeated the analyses after exclusion of pregnancies with such complications. In addition, the large data set in Paper I, also enabled us to study smoking and risk of preterm birth after stratification for gestational age and onset of labour.

In Paper III, it was possible to analyse a small subgroup, pregnancies with placental abruption, and risk of perinatal deaths in this cohort, and to make stratification for preterm and term births. Paper I and III are based on practically all pregnancies in Sweden. However, selection bias may still be a concern, since women with missing information on smoking had higher rates of preterm births and placental abruption. A limitation is that the studies were restricted to information and potential confounders included in the register. Thus, we cannot exclude the possibility of residual confounding. Another potential limitation is that there might be variation of quality and misclassifications in the register that you can not control. These limitations should generally with respect to the studied outcomes, if anything, lead to underestimation of risks.

Patient records

Information in patient records are often more complete than in the MBR, and indistinct data can be checked. This makes it possible to obtain a more precise and complete classification of, for example, causes of preterm birth. A study based on information retrieved directly from patient records, makes it possible to compensate for some of the weaknesses in register-based studies. Information and diagnoses can be checked for accuracy in the written patient reports. Then misclassification and errors in forwarding of data to the large register is avoided. Covariates that are not forwarded to the MBR can be extracted. But data in the patient records is not always complete, since they may be filled in incompletely and patient history not adequately documented. The process of retrieving information from patient records is very time

consuming, and the number of records and information that are possible to handle is limited, which restricts the sample size.

In Paper II, we used a case control-study, nested within a population-based cohort, which is a good choice for studying a proportionately rare outcome, such as very preterm birth (Hennekens and Buring 1987; Beaglehole R 1995). To avoid socially uneven recruitment, the cohort included all delivery records on very preterm birth from two big hospitals situated in socially different areas. Information was prospectively recorded in all records, which precludes recall and selection bias in that process. As the recruitment of the control group followed the registration-time of the cases, differences should not be dependent of uneven recruitment of the studied groups. To minimize observation bias in retrieval of data for the study, the records were systematically surveyed according to a structured standardized protocol, which also ensured high accuracy of defining cases and controls. A strength of this study is that information of gestational age, smoking, onset of labour and covariates are more complete compared to a study based on a large register such as the MBR. Presence of information on smoking habit and several covariates was high, but not complete. For height and length the proportions of missing data were substantial because of a defective entry of data into the patient records. Since rates of missing information on smoking and other covariates were generally similar among cases and controls, it is unlikely that this introduced a selection bias. However, the reduced number of cases and controls with complete information will of cause negatively influence the power in the multivariate analyses. There is also a possibility of residual confounding, since the records did not include information about a number of potentially interesting covariates, such as smoking habit later in gestation, other drugs, nutritional data, working conditions and other potentially stressful events. Another limitation was that some of the analyses were also hampered by low number of cases.

Ultrasonic measurements

Since the fetal blood pressure measurements and other direct examinations of the fetus are impossible to perform, other methods for estimating fetal blood flow regulation and vessel properties have been developed. The ultrasonic tracking system that continuously monitors diameter changes of a vessel segment, used in the Paper IV study, provides a possibility of non invasive assessment of mechanical properties of the fetal arteries. The technique has a high temporal and spatial resolution which is necessary because of the small pulsatile diameter changes in the fetal aorta (Lindstrom K, Gennser G et al. 1987; Benthin, Dahl et al. 1991)

The technique is not suitable for clinical praxis as it is time consuming. The fetus and mother have to be resting and insonation must be optimal. Deviation of the probe from the optimal position produces a rapid decline in image quality, with loss of echoes from the vessel walls, along with the trackers reduced ability to lock on to the walls. During measurement the target is continuously displayed on the scanner screen, which exposes tilting of the probe and allows its correction (Manor, Dahl et al. 1993). Gross limb movements and breathing trunk movements of the fetus should not take place since that disturb the measurements (Hu, Nijhuis et al. 1997).

To avoid operator errors the measurements should be made by the same trained investigator. In the Paper IV study ultrasonic examination was performed by one of the authors, dr J Hu who in a previous study had performed measurements with intra-observer variability which, expressed as the coefficient of variation, was 9.3% for the PWV, 4.2% for the Dd, and 2.9% for ΔD (Hu, Nijhuis et al. 1997). The technique of non-invasive monitoring of the pulsatile diameter changes and of the pulse wave velocity (PWV) in the fetal aorta has previously been evaluated

and described in similar studies (Sindberg Eriksen and Gennser 1984; Lindstrom K, Gennser G et al. 1987; Stale, Marsal et al. 1991; Hu, Nijhuis et al. 1997; Gardiner, Brodszki et al. 2001).

In Paper IV all cases were documented smokers, not only by self documentation, but also by control of serum cotinine, and according to that the groups were well defined. In studies where information is retrieved from patient records or the MBR you have to rely on self documentation of smoking habits obtained at the first visit to the antenatal clinic. In such studies there will always be uncertainty whether some women might have changed their smoking habit later in pregnancy (Haddow, Knight et al. 1987).

To limit the differences between the groups as much as possible with regard to the smoking habit, the participants had to be healthy without pregnancy complications and matching was made for maternal and gestational age. Later development of pregnancy complications caused exclusion. The study however, suffers from wide ranges and there are other inter-individual differences that were not controlled for, such as parity, maternal height, nutritional status, socio-economic factors, stress and genetics. A way of handling these problems had been to perform repeated measurements in each subject where the cases to some extent could have been their own controls.

Loss of data because of unacceptable pulse wave curves in the present study was limited almost only to the smokers and especially to curves for PWV calculations. It is more difficult to get acceptable curves for calculating PWV since it is necessary to get two pulse wave traces recorded simultaneously at two sites of the vessel. For calculating the other parameters of diameter changes a trace from one of the trackers is enough. A difficulty among the smokers was that the fetuses seemed more active and thus more difficult to examine. This might be interpreted as nicotine abstinence. It is observed that there is an increased risk for hyperkinetic disorder in children of mothers who smoked during pregnancy (Linnet, Wisborg et al. 2005).

External validity

Internal validity is the lack of systematic and random errors in a study and external validity expresses the generalizability of the results. There is often a trade-off between internal and external validity. For example, a homogeneous population favours internal validity, but the findings may not be relevant for other populations. In etiological studies, one should favour internal validity - if the internal validity is low, the results can hardly be transferred to other populations.

In Paper I and Paper III the analyses are based on samples including practically all pregnancies in Sweden during 1991-93 (Paper I) and 1987-93 (Paper III). In both studies, we were able to perform stratified analyses and to control for confounders included in the MBR. Thus, both internal and external validity should be considered as high.

In Paper II, the fairly complete data set and adjustment for confounding factors strengthen the internal validity, while the small sample size in some subgroups lowers the internal validity. The study included deliveries from two large hospitals in Stockholm, covering socially different populations. Since it is uncertain whether results from a study performed in Stockholm can be generalized to the rest of Sweden, this lowers the external validity.

In Paper IV the internal validity is reduced because of non optimal recordings in some of the smokers, wide ranges of obtained parameters, and a small study group in which measurements are not repeated later in pregnancy. Matching and exclusion of pregnancy complications, to

reduce the difference between the studied groups to the smoking habit, strengthen the internal validity. Further studies are needed to find out if the results may be reproduced and generalized.

The cause-effect relation

In observational studies, we cannot prove causal associations between exposure and the likelihood of outcome. However, the following criteria have to be considered when estimating causality(Hennekens and Buring 1987; Beaglehole R 1995):

- Time-sequence: the exposure precedes the outcome by a period of time that is consistent with a proposed biological mechanism.
- Consistency: there are similar or supportive findings in other studies from independent populations.
- Strength of association: the higher relative risk of an association, the more likely is a true cause-effect relation of exposure and outcome.
- Dose-response relationship: the risk for the proposed outcome increases with exposure.
- Reversibility: the risk for the outcome decreases or disappears when the exposure is removed.
- Biologically plausibility: there are postulated biological mechanisms by which the exposure might reasonably influence the risk for the outcome.

Maternal smoking and risk of preterm birth (Papers I and II)

We found maternal smoking to be a risk factor for preterm birth (Papers I and II). A supposed relation between smoking and preterm birth was early pointed out in reports from the 1950's and 1970's (Simpson 1957; Meyer and Tonascia 1977). In later studies, some studies confirmed this finding (Shiono, Klebanoff et al. 1986; Wen, Goldenberg et al. 1990; Kramer, McLean et al. 1992; Wisborg, Henriksen et al. 1996), whereas others did not (Adams, Sarno et al. 1995; Zhang and Bracken 1995; Mercer, Goldenberg et al. 1996; Nordentoft, Lou et al. 1996). However, large epidemiological studies consistently report that maternal smoking is a risk factor for preterm birth (Cnattingius, Forman et al. 1993; Meis, Michielutte et al. 1995; Chan, Keane et al. 2001).

We found that smoking was associated with a higher risk in very preterm birth (≤32 weeks) compared to moderate preterm birth (33 to 36 weeks). In heavy smokers we found adjusted OR 1.6 (95%CI 1.4-1.8) and 1.4 (95%CI 1.3-1.4), respectively (Paper I). The magnitude of these risks are in accordance with earlier reports (Meyer and Tonascia 1977; Shiono, Klebanoff et al. 1986), and has been confirmed later (Morken, Kallen et al. 2005).

It has been discussed whether the effect of smoking on preterm birth exerts its effect through pregnancy complications associated with smoking *and* preterm birth, and/or also has an effect on spontaneous preterm labour (Meyer and Tonascia 1977; Harlow, Frigoletto et al. 1996) We found that maternal smoking had a stronger effect on spontaneous compared to induced preterm births (Paper I), which is supported by other reports (Meis, Michielutte et al. 1995; Morken, Kallen et al. 2005). The smoking-related risk remained essentially unchanged when we excluded pregnancy complications associated with smoking (placental abruption, placenta praevia, preterm PROM, IUGR and hypertensive diseases), further suggesting that smoking may be directly associated with preterm labour.

In addition, we were able to confirm previous observations that smoking was associated with a dose-dependent increase in risk of preterm birth (Paper I and II) (Meis, Michielutte et al. 1995; Wisborg, Henriksen et al. 1996; Burguet, Kaminski et al. 2004) Moreover, a reduction in risk of preterm birth has been reported in women who quit smoking during the first trimester (Mainous and Hueston 1994). In women who stop smoking from one pregnancy to another the risk of preterm birth will be reduced to that of non-smokers (Cnattingius, Granath et al. 1999).

We investigated the risk of very preterm birth by cause, and found that there was a dose-dependent association between maternal smoking and risk of very preterm birth caused by preterm labour (Paper II). This was also recently shown by Burguet et al. (Burguet, Kaminski et al. 2004), but we were also able to demonstrate that maternal smoking is probably also a risk factor for 'idiopathic' preterm labour, after excluding cases with infections, conisation of the cervix, hydramniosis, uterine and fetal anomalies. This further supports the suggestion that smoking may be directly associated with the initiation of preterm labour.

In the Paper II study we found that smoking also exerts its effect on very preterm birth through late pregnancy bleedings (placental abruption and placenta praevia) and probably through preterm PROM, confirming results from previous investigations (Spinillo, Capuzzo et al. 1994; Castles, Adams et al. 1999; Burguet, Kaminski et al. 2004).

Maternal smoking and risk of placental abruption (Paper III)

We found maternal smoking to be a risk factor for placental abruption, which has been repeatedly reported in previous studies (Raymond and Mills 1993; Spinillo, Capuzzo et al. 1994; Ananth, Savitz et al. 1996; Kramer, Usher et al. 1997).

We also showed that maternal smoking was dose-dependently associated with an increase of placental abruption. Adjusted OR for moderate and heavy smokers were 1.8 (95% CI 1.8-2.1]) and 2.2 (95% CI 2.0-2.5), respectively (Paper III). This relatively strong risk for placental abruption in maternal smoking is not always found in previous studies, but in a meta-analysis pooled OR was 1.9 (95% CI 1.8-2.0) and dose-dependency was also indicated (Ananth, Smulian et al. 1999). It is also reported that the duration of smoking is associated with an increased risk of placental abruption and placental infarcts, and that women who stop smoking during pregnancy have a lower risk of placental abruption (Naeye 1979; Naeye 1980).

In addition, when we studied the risk of perinatal death in pregnancies complicated with placental abruption, we found that smoking was associated with dose-dependent increased risks of perinatal death in both preterm and term pregnancies, with slightly higher risk in term births. Earlier studies about perinatal death in cases of placental abruption report increased risk of perinatal death among smokers (Meyer and Tonascia 1977; Raymond and Mills 1993; Tuthill, Stewart et al. 1999). Thus, this suggests that some of the smoking related perinatal mortality may be attributed to smoking related perinatal death in pregnancies with placental abruption. In addition, results from a Swedish study suggest that the smoking related risk of stillbirth is entirely explained by fetal growth restriction and placental complications (Raymond, Cnattingius et al. 1994). It has previously also been reported that women who stop smoking during pregnancy not only reduce the risk of placental abruption, but also the risk of perinatal mortality due to placental abruption (Naeye 1980).

We also found the risk of perinatal death increased in smokers without placental abruption (Paper III). This corroborates with studies that has reported maternal smoking to be a risk

factor for unexplained perinatal death and for preterm and unexplained stillbirth (Tuthill, Stewart et al. 1999; Froen, Arnestad et al. 2001; Stephansson, Dickman et al. 2001).

Biological plausibility and the association of maternal smoking to preterm birth and placental abruption. (Papers I, II and III).

There is strong evidence that maternal smoking has an effect on preterm birth and placental abruption, but the biological mechanisms are unclear. Probably maternal smoking might influence the initiation of preterm birth and placental abruption in many ways through infectious/inflammatory, vascular and stress related pathways (for a general discussion of these pathways see page 16 and 20).

Smoking and spontaneous preterm birth

Spontaneous preterm birth starts with spontaneous labour or rupture of the membranes (preterm PROM). Spontaneous preterm birth can be finished by spontaneous vaginal delivery, instrumental vaginal delivery or Caesarean section. Causes of spontaneous preterm birth are spontaneous labour and preterm PROM.

We found that maternal smoking increased the risk of preterm birth more in spontaneous, compared to induced preterm birth, especially in very preterm birth (Paper I). Infection and inflammation is postulated to be an important pathway for spontaneous preterm birth, and the proportion grows with degree of prematurity (Andrews, Hauth et al. 2000; Challis, Lye et al. 2001; Goepfert, Goldenberg et al. 2001).

Both clinically evident and clinically silent intrauterine infections may influence the risk of spontaneous preterm birth (Romero, Sirtori et al. 1989; Romero, Gomez et al. 2001). With the entry of bacteria into the decidua, leukocytes are recruited and cytokine production follows, which triggers the prostaglandin synthesis (Keelan, Blumenstein et al. 2003). Smoking might be associated with vaginal infections, and is reported to have a dose-response relationship to the presence of bacterial vaginosis. Bacterial vaginosis is also related to the risk of chorioamnionitis, suggesting that bacterial vaginosis in smokers might be a mediator for preterm birth (Hillier, Nugent et al. 1995; Andrews, Hauth et al. 2000; Hellberg, Nilsson et al. 2000; Challis, Lye et al. 2001).

Cytokines that increase in infection and inflammation also stimulate metalloproteinases that are involved in cervical ripening and degradation of the fetal membranes (Keelan, Blumenstein et al. 2003). This process favoures rupture of membranes. With regard to preterm PROM, changes in collagen types in prematurely ruptured membranes have been reported (Kanayama, Terao et al. 1985). We found that maternal smoking is probably associated with preterm PROM (Paper II), a finding which is supported by results from several other studies (Castles, Adams et al. 1999; Lee and Silver 2001; Burguet, Kaminski et al. 2004). Ascorbic acid is necessary for the maintenance of collagen and for collagen synthesis and smokers seem to have a changed nutritional status with lowered ascorbic acid levels in serum and in amniotic fluid (Barnes 1975; Barrett, Gunter et al. 1991; Faruque, Khan et al. 1995). This suggests that smoking also may increase the risk of preterm PROM through compromising of the integrity of the membranes, and not only through infection/inflammation.

Potentially important infections include not only genital, but also non-genital maternal infections such as urinary tract infections, pneumonia, malaria and typhoid fever. But antibiotic treatment has dramatically reduced preterm birth related to these infections (Romero, Gomez et al. 2001). Periodontitis is more common among smokers compared to non-smokers and

recently, periodontitis has been proposed as a risk factor for preterm birth. (Offenbacher, Lieff et al. 2001; Jeffcoat, Hauth et al. 2003). In these cases, there is probably a haematogenous dissemination of cytokines and/or bacteria through the placenta. A chorioamnionitis may occur as a sign of microbial infection, but may also appear as an aseptic inflammation where the tissue is more susceptible to bacterial invasion.

We found that maternal smoking increased the risk for preterm labour and 'idiopathic' preterm labour (Paper II). We suggest that smoking may make the tissues more sensitive to labour stimulating agents through inflammatory changes (Holt 1987). In response to maternal smoking, indications of inflammation in the feto-placental unit and the neonate have been reported (Naeye 1978; Narahara and Johnston 1993; Beratis, Varvarigou et al. 1999; Noakes, Holt et al. 2003). Nicotine seems to influence all aspects of the immune system and smoking is shown to be associated with elevated cytokines in healthy females (McAllister-Sistilli, Caggiula et al. 1998; Bermudez, Rifai et al. 2002). Smoking might be a mediator in aseptic inflammation that may facilitate periodontitis, chorioamnionitis and cervicitis, which in turn may be a basis for the development of preterm labour. Another report that augments the evidences of a labour favouring effect of smoking is that not only nicotine but also cotinine might interfere in the balance of activators and inhibitors of the myometrium. This probably occurs by activating formation of prostaglandins in villous tissue and amniotic membranes (Rama Sastry, Hemontolor et al. 1999).

There are also other ways smoking might influence the initiation of preterm labour, for instance through stress. Recently, more attention has been paid to stress as a risk factor for preterm birth. In stressful situations CRH that interacts with prostaglandins and oxytocin, might influence the balance of inhibiting and activating factors (Kramer, Goulet et al. 2001). Maybe smoking influences the cortisol-induced feedback on placental CRH, since there are reports about elevated cortisol levels in adult smokers, and also in amniotic fluid (Lieberman, Torday et al. 1992; Pomerleau 1992). In a stressful situation you often hear about premature contractions, and in animal experiments smoking is reported to enhance expression levels of the oxytocin-receptor mRNA and to increase the sensitivity of oxytocin in rat myometrial tissue (Egawa, Yasuda et al. 2003)

Smoking and induced preterm birth

In induced or indicated preterm birth, the delivery takes place before start of labour or preterm PROM according to the physician's decision for maternal or fetal medical causes. Causes of induced preterm birth are late pregnancy bleedings (placental abruption and placenta praevia), hypertensive diseases (including preeclampsia), IUGR and other various causes.

We found that maternal smoking had a weaker association to induced compared to spontaneous preterm birth (Paper I). This might be explained by the diverse relation of smoking to the causes of induced preterm birth. Maternal smoking is consistently shown to be a risk factor for placental abruption, placenta praevia and IUGR (Naeye 1978; Naeye 1980; Wen, Goldenberg et al. 1990; Ananth, Savitz et al. 1996; Castles, Adams et al. 1999; Kallen 2001). Paradoxically, maternal smoking is also shown to reduce the incidence of hypertensive diseases during pregnancy (Cnattingius, Mills et al. 1997; England, Levine et al. 2002). Impairments in the vascular bed might constitute a common aetiology for these complications in spite of their differences in relation to smoking.

We found maternal smoking to be a risk factor for placental abruption (Papers II and III). Vascular lesions in the placenta have been reported in connection with preterm birth and

placental abruption (Naeye, Harkness et al. 1977; Germain, Carvajal et al. 1999). Placentation might be affected by maternal smoking since inhibition of early cytotrophoblast differentiation is reported in smokers, and also nutritional adversities such as lower serum folate and ascorbic acid levels (Barrett, Gunter et al. 1991; McDonald, Perkins et al. 2002; Genbacev, McMaster et al. 2003). Placental histopathology is seen in smokers and stem villous arteries of heavy smokers are reported to have altered mechanical properties and a greater vasoconstrictive response to endothelin 1 than nonsmokers (Naeye 1978; Naeye 1979; Asmussen 1980; Clausen, Jorgensen et al. 1999). How these changes in the placenta might affect the timing of labour and placental abruption is unclear. Possibly preterm labour and placental abruption partly are two different expressions of a reaction to placental lesions sharing aetiology.

SGA, preterm birth, placental abruption and pregnancy-induced hypertensive diseases are shown to increase the risk of placental abruption in subsequent pregnancy (Karegard and Gennser 1986; Rasmussen, Irgens et al. 1999). This suggests that these pregnancy outcomes/complications share an aetiological factor or may represent different clinical expressions of recurring placental dysfunction. Decidual necrosis at the margin of the placenta, placental infarcts, hypovascular atrophic villi and other changes are shown to be more common in smokers than in non-smokers, and such placental impairment could serve as weak points predisposing for premature separation of the placenta (Naeye 1979; Naeye 1980; Mochizuki, Maruo et al. 1984).

Vitamin C is important for the maintenance of collagen and vascular structure, and smokers seem to have lower ascorbic acid levels in serum and amniotic fluid compared to non-smokers (Barnes 1975; Barrett, Gunter et al. 1991). If the placental vascular tissue looses some of its strength, the risk of bleeding and placental abruption might increase. Smokers have also an altered output of fibronectin (an adhesion promoting protein) in amnion and placenta compared to non-smokers, but the significance of this finding is unclear (Shimizu, Dudley et al. 1992).

We also found maternal smoking to be a risk factor for perinatal death in pregnancies with placental abruption and to a less extent in pregnancies without placental abruption (Paper III). Smoking-induced impairment of the placenta may reduce blood perfusion, and will, together with an increased incidence of IUGR and prematurity, probably make the fetus and neonate more vulnerable, especially in combination with bleeding that can be excessive in placental abruption (Asmussen 1980; Andersen and Hermann 1984; Kramer, Usher et al. 1997). In stillbirth related to smoking, fetal growth retardation is more common in preterm, compared to term births (Gardosi, Mul et al. 1998).

In summary:

- The association of maternal smoking and preterm birth fulfil all the criteria of a causal relationship, except for the strength of the association, which is modest.
- The association of maternal smoking and preterm PROM did not fulfil the criteria of significant association in Paper II, and reversibility need to be addressed. However, results from previous reports and biological plausibility favour a causal relationship.
- The association of maternal smoking and preterm labour fulfil all the criteria of a causal relationship.
- The association of maternal smoking and placental abruption fulfil all the criteria of a causal relationship.
- The association of maternal smoking and perinatal death in pregnancies with placental abruption fulfil the criteria of a causal relationship, although the association is modest.

Smoking and pulse waves changes in fetal aorta (Paper IV)
A study of the effect of chronic smoking on pulse waves in the fetal aorta has not previously been carried out, which prevents comparison with similar investigations.

PWV is dependent on blood pressure and vessel stiffness and increases with ageing (Lindstrom K, Gennser G et al. 1987; Cheng, Baker et al. 2002). PWV is generally reported to increase also during pregnancy (Sindberg Eriksen, Gennser et al. 1984; Hu, Bjorklund et al. 1998; Gardiner, Brodszki et al. 2001), but not always (Stale 1991). In the study by Gardiner et al. 20 normally growing fetuses with normal amount of amniotic fluid and normal umbilical artery Doppler measurements, were examined monthly from a gestational age of 20 weeks to term. However, there is no information about potential covariates, such as smoking and hypertensive diseases (Gardiner, Brodszki et al. 2001). In our study we found PWV to increase with gestational age among smokers, but not among non-smokers. Possibly the absence of pregnancy complications and other disorders that might influence the vascular bed favour this result. Our earliest measurements were made later in pregnancy, in the 31st gestational week, which may have reduced the possibility of getting a significant increase of PWV in non-smokers. Although there was a significant difference between smokers and non-smokers with regard to the relation of PWV to gestational age, the role of chance cannot fully be ruled out, as the measurements were not longitudinal and inter-individual variability was considerable.

Another way of estimating stiffness in arteries is strain, which is supposed to decrease during gestation (Sindberg Eriksen, Gennser et al. 1984; Mori, Trudinger et al. 1997). In our study strain decreased in both groups, but significantly only among smokers, but interaction analysis did not reveal any difference between the groups.

MIV increased among the non-smokers, which is congruent with most other investigations of normal fetuses (Stale 1991; Hu, Bjorklund et al. 1998; Gardiner, Brodszki et al. 2001), but not in all (Sindberg Eriksen, Gennser et al. 1984). In our study, MIV did not change during gestation among smokers, and there was a significant difference between the change of MIV in the groups.

PWV and MIV are obtained in different ways from the pulse waves and do not have the same determinants. As the results pointed in the same direction, this strengthens the possible effect of smoking.

Changes of pulse waves in fetal aorta and biological plausibility of relation to smoking habit (Paper IV)
The long-term effects on arterial elastic properties of chronic smoking in adults are partly unclear, but increased PWV in both normotensive and hypertensive smokers has been reported (Levenson, Simon et al. 1987). The arterial wall thickens and stiffens with age, and smoking seems to exaggerate this process (Liang, Shiel et al. 2001; Cheng, Baker et al. 2002). In atherosclerotic arteries of adult smokers, increased collagen content and reduction of elastin are shown (Ribeiro, Jadhav et al. 1983; MacSweeney, Powell et al. 1994). In the fetus, intimal preatherosclerotic thickening in coronary arteries is reported in cases with smoking mothers (Matturri, Lavezzi et al. 2003), and in the placenta of smokers elevation of collagen is shown (Asmussen 1980; Andreucci, Teodoro et al. 1992). There is a high correlation between the mechanical function and the disposition of collagen and elastin in the arterial wall (Dobrin 1978; Glagov, Vito et al. 1992).

Nicotine administration in term pregnant sheep is reported to produce significant increases in fetal arterial blood pressure and umbilical vascular resistance (Clark and Irion 1992).
Such a repeated process in daily smoking may be a plausible pathway for arterial wall remodelling and stiffness change (Folkow 1987). Thus, a smoking related increased fetal arterial stiffness might be a plausible finding, and may explain a difference in PWV between smokers and non-smokers.

Recently, it was reported that maternal cigarette smoking probably is associated with evidence of chronically increased resistances in the uterine, umbilical and fetal middle cerebral arteries. In addition, a dose response relationship of smoking to the systolic/diastolic (S/D) ratio in the fetal middle cerebral artery was found (Albuquerque, Smith et al. 2004). It is also shown that there is a dose-dependently reduced prostacyclin-like activity in umbilical arteries associated with maternal smoking (Ahlsten, Ewald et al. 1990). Reduction of blood flow through the placenta and increased blood flow in the abdominal fetal aorta is also reported among habitual smokers (Andersen and Hermann 1984; Eldridge, Berman et al. 1986). If smoking results in an increased total peripheral resistance, MIV might not increase during gestation as expected. In fetuses of mothers with insulin dependent diabetes, no change of MIV during gestation is reported. Maternal smoking might have a similar impact on the peripheral resistance as diabetes (Hu, Bjorklund et al. 1998).

In summary:
Causal associations of maternal smoking to changes in pulse wave characteristics may be questioned, since the strength of the associations shown in our study is weak, and there are no other similar studies. However, there seems to be a biological plausibility of the findings. The study was too small to study dose responsiveness, and the reversibility of exposure is not an applicable criterion of causality in the study. Further studies are needed to clarify if there is a causal relationship between maternal smoking and fetal pulse wave characteristics.

Implication of findings and health perspectives

Maternal smoking habit was found to be a risk factor for preterm birth, especially very preterm birth. Preterm birth is the main health problem in children, threatening all racial and ethnical groups, but particularly minority populations and the poor (Goldenberg and Rouse 1998; Mattison, Damus et al. 2001). About half of the neurodevelopemental problems can be ascribed to preterm birth, and nearly half the charges for US infant hospitalization in 2002 were related to preterm birth (Goldenberg 2002; Green, Damus et al. 2005). Very preterm birth is responsible for the largest part of perinatal morbidity and mortality in preterm birth (Goldenberg and Rouse 1998; Slattery and Morrison 2002).

Extensive research has been carried out in order to find biomarkers for prediction of preterm birth most of them have poor predictive value (Goldenberg 2002). Most intervention programmes, aiming to reduce the incidence of preterm birth, have not been successful (Main, Gabbe et al. 1985; Bryce, Stanley et al. 1991; Collaborative-Group 1993; Ananth, Joseph et al. 2005). Smoking is a risk factor that is easy to define, reducible and easy to explain. A Cochrane meta-analysis has shown that smoking cessation programmes in pregnancy reduce the proportion of women who continue to smoke, and reduce the incidence of low birth weight and preterm birth. However, the pooled trials had inadequate power to detect reductions in perinatal mortality or very low birth weight (Lumley J 2004).
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Maternal smoking was also found to be a risk factor for placental abruption, and perinatal death in pregnancies complicated with placental abruption. This is of major importance, since we found that the perinatal mortality rate in pregnancies with placental abruption was as high as 10.6%. Both placental abruption and perinatal mortality are important factors in preterm birth (Ananth, Berkowitz et al. 1999). There are no programmes for prevention of placental abruption, and predictive markers of imminent placental abruption are elusive. There is a tendency to repeat placental abruption in successive pregnancies. After a delivery with placental abruption, antenatal control in subsequent pregnancy therefore often comprises closer antenatal surveillance. However, it is not known whether this has any positive effect on the outcome, and monitoring up to three months before the gestational age of the initial abruption is reported to be necessary (Rasmussen, Irgens et al. 2001). Smoking cessation seems to be the only preventive action with reported effect, and increased awareness of the smoking related risk of placental abruption is essential (Naeye 1980).

Most women in the developed countries know that cigarette smoking is harmful in pregnancy, but knowledge about specific smoking-related risks during pregnancy is often deficient (de Vries and Backbier 1994; Roth and Taylor 2001; Lendahls, Ohman et al. 2002). In a small study from South Africa on coloured women, where 42.5% of the pregnant women were smokers, 8.2 % thought smoking was harmless, 62% new about risk of disturbance of fetal growth, and around 10% had knowledge about a few other harmful effects in pregnancy (Viljoen and Odendaal 2005).

However, pregnancy seems to be the occasion in life when most women quit smoking, and probably are most receptive to information about the harmful effects of smoking. In Sweden about 50% quit smoking when they get pregnant, and in the small study from South Africa they reported that about 34% of the ex-smokers had stopped when they got pregnant (SoS 2005; Viljoen and Odendaal 2005). Presently, we do not know whether more knowledge about the specific smoking related risks would increase the motivation of smoking cessation in pregnancy in developed countries. In developing countries, where lack of knowledge about smoking related adverse effects in pregnancy is more common, information campaigns may be more effective.

Harmful effect of smoking in pregnancy is not questioned but there is an ongoing debate whether this effect might extend to infancy and further to adult life. Most women who smoke during pregnancy continue to smoke after birth and expose the child to the risks of passive smoking. There may be difficulties in separating probable long term effects of maternal prenatal smoking from effects of passive smoking after birth. SIDS has been discussed in this context, but most likely both prenatal and postnatal smoking exposure increase the risk of SIDS (Cnattingius 2004). It is also reported that maternal smoking during pregnancy is related to infant overweight independently of IUGR (Wideroe, Vik et al. 2003). The conclusion in this study was that the effect may be attributed to specific effects of maternal prenatal smoking. This supports the hypothesis of 'fetal origin of adult disease', and the suggestion that fetal exposure to smoking might be an etiological factor in subsequent development of cardiovascular diseases.

Maternal smoking probably affects mechanical properties of the fetal aorta. In the present study the relations of PWV and MIV to gestational age in smokers differed from that in non-smokers. One explanation of the diversity between smokers and non-smokers, might be increased resistance in the placenta in the smoking group as well as decreased aortic elasticity or/and increased fetal blood pressure. If such circumstances might be of importance after birth is not

known. There are reports about elevated blood pressure in infants of smoking mothers, but the results are inconsistent (Beratis, Panagoulias et al. 1996; O'Sullivan, Kearney et al. 1996; Ley, Stale et al. 1997; Blake, Gurrin et al. 2000; Browne, Colditz et al. 2000). Information about blood pressure in adults in relation to maternal smoking habits during pregnancy is lacking.

Low birth weight, which is a known risk of maternal smoking, is reported to be a risk factor of hypertension and cardiovascular disease in adult life (Gennser, Rymark et al. 1988; Barker, Winter et al. 1989; Martyn, Barker et al. 1995; Kallen 2001). In a report about elevated blood pressure in the offspring of smoking mothers, the association is reported not to be wholly attributed to low birth weight (Blake, Gurrin et al. 2000). In adults, each smoked cigarette causes an instant decrease in arterial elasticity and a transient rise in blood pressure. The feto-placental vascular unit also seems to react to maternal smoking with repeated vessel constriction (Lehtovirta and Forss 1978; Sindberg Eriksen and Gennser 1984; Lambers and Clark 1996; Benetos, Waeber et al. 2002). Such a repetitive process may in conjunction with already increased placental resistance in smokers, be one biologically plausible pathway for development of elevated blood pressure *in utero*. In case of an intrauterine rise in blood pressure, prerequisites for a remodelling of the arterial wall composition are at hand, and may trigger a course of a subsequent progressive amplification of blood pressure (Berry 1978; Folkow 1987; Law, de Swiet et al. 1993; Martyn, Barker et al. 1995). Hypothetically, such a process might be a basis for development of cardiovascular disease in adult life.

Maternal smoking not only affects the health of the smoker but also the pregnancy, the infant and probably the offspring health in the long run. To this should be added the effect of passive smoking and economic costs for the family and society. In the book from WHO "Women and the Tobacco Epidemic, Challenges for the 21st Century" 2001 it is stated that the epidemic of tobacco use is a woman's issue (WHO 2001). In contrast to men, tobacco use is rising among women, especially among young women and in women in developing countries. Currently about 200 million women smoke, and it is estimated that by 2025 there will be around 530 million female smokers, unless new innovative, robust initiatives are taken (Sarnet JM 2001). The tobacco companies are active on the new markets and introduce new brands and carry out campaigns addressed specifically to women. The need of gender-specific anti-tobacco programmes has been recognized by WHO (Amos and Haglund 2000; Christofides 2001; Kaufman and Nitcher 2001). Although Sweden early made an effort to address women, smoking is presently in Sweden more prevalent among women than men (FHI 2005). The prevalence of smoking among 18-24 year old women in Sweden has decreased to 16% the last years (in young men 8%). However, the smoking prevalence among young women who get pregnant is still far too high (SoS 2005).

Future perspectives

The issue of effectiveness of smoking cessation programmes addressed to women is crucial. There is a need for studies revealing what kind of information that can further decrease the smoking prevalence. The relation of birth outcome in preceding pregnancies to maternal smoking habits in successive pregnancies would be of interest. Mapping of the knowledge of the smoking related pregnancy complications and offspring risks in relation to amount smoked and to cessation in pregnancy would also be of interest.

We have studied smoking in pregnancy and there are still conditions that are not clarified.

We know that perinatal mortality is high in pregnancies with placental abruption, and that fetal growth restriction and gestational age play important roles. To study the relation of maternal smoking to perinatal mortality by cause of birth, and to gestational age, would be of interest. In addition, another question is to what extent fetal growth restriction explains possible associations between smoking and causes of perinatal mortality.

The effect of maternal smoking on the cardiovascular health of the offspring is not much studied. We know that being born with low birth weight increases the risk for hypertension and cardiovascular disease in adult life, but studies of these outcomes in adults in relation to maternal smoking during the fetal period are lacking.

Another field of interest is further studies of mechanical properties of the fetal aorta in smokers. It would be of interest to study pulse wave characteristics of the fetal aorta in smokers and non smokers, followed by an investigation of the endothelial function in the infants after birth. In addition, a study of collagen/elastin distribution of the umbilical arteries would be interesting.

MAIN CONCLUSIONS OF THE THESIS
(With the conclusions, the aims are repeated in italics)

- Maternal smoking is a risk factor for preterm birth. The risk is higher in spontaneous compared to induced preterm births. The risk is also higher in very preterm birth compared to moderately preterm birth.
- Maternal smoking is a risk factor for preterm labour and probably also for 'idiopathic' labour in very preterm birth. Maternal smoking was also shown to increase the risk of very preterm birth caused by pregnancy bleedings, and probably also for very preterm birth caused by preterm PROM

  To study maternal smoking in relation to preterm birth
  a) Is smoking a risk factor for preterm birth?
  b) What is the relation between smoking and onset of labour in preterm birth?
  c) What is the relation between smoking and gestational age in preterm birth?
  d) What is the relation between smoking and different causes of very preterm birth?

- Maternal smoking is a risk factor for abruptio placentae.
- Maternal smoking is a risk factor for perinatal death in pregnancies without placental abruption.
- Maternal smoking is a risk factor for perinatal death among women with placental abruption, and the risk seems to be higher in term birth.

  To study the relation of maternal smoking to placental abruption
  a) To estimate the smoking-related risk of placental abruption;
  b) Is smoking a risk factor for perinatal death in pregnancies without placental abruption?
  c) What is the relation of smoking to perinatal mortality in pregnancies with placental abruption?

- Maternal daily smoking possibly influence pulse wave characteristics in the fetal aorta.

  To study the relation of daily maternal smoking to pulse wave characteristics in the fetal aorta.

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