Thesis for doctoral degree (Ph.D.) 2008

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INFECTIONS AND INFECTIOUS RELATED MORBIDITY DURING PREGNANCY – SHORT AND LONG-TERM EFFECTS

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Stockholm 2008

Published by Karolinska Institutet.

ISBN 978-91-7409-237-0

Printed by
REPROPRINT AB
Stockholm 2008
www.reproprint.se
Gårdsvägen 4, 169 70 Solna

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ABSTRACT

The aim of this thesis was to elucidate the association between infections during pregnancy and risks of miscarriage, preterm delivery and later development of autism.

We identified 60 755 women with singleton births between 1973 and 2000 in the counties of Uppsala and Gävleborg who had a Pap smear taken during pregnancy. Risk of adverse pregnancy outcome with respect to signs of infection on Pap smear was calculated in logistic regression models. Presence of Coccobacilli on Pap smear slightly increased the risk of delivery of an SGA infant and presence of *Trichomonas* increased the risk of moderately preterm delivery (32-36 weeks). When the analyses was restricted to Pap smears taken within four weeks before delivery we found that presence of Coccobacilli was associated with a fourfold increase in risk of very preterm delivery (≤31 weeks).

Among pregnant women in Stockholm County, we identified 235 cases with second trimester miscarriage, 269 cases with very preterm delivery and 301 controls with term delivery for which we had archived blood samples for Rubella serology screening in early pregnancy. Blood samples were analyzed for Parvovirus B19 and Herpes viruses. Viremia was found in 11 (4.7%) women with second trimester miscarriage and 10 (3.7%) women with very preterm birth, compared to 5 (1.7%) women who delivered at term, corresponding to adjusted odds ratios (95 percent confidence interval) of 3.32 (0.93-11.8) and 2.21 (0.71-6.84), respectively.

A cohort of all primiparous women with live singleton births from 1987 through 2000 in Sweden (n= 601 883) was linked to the Swedish Hospital Discharge Register to obtain information on previous pregnancy losses. The risk of extremely preterm delivery (≤ 27 weeks), very preterm delivery (28-31 weeks), and moderately preterm delivery (32-36 weeks) associated with previous pregnancy loss was estimated in logistic regression models. Previous spontaneous abortions and previous missed abortions were associated with increased risks of preterm delivery, and the risks increased with severity of preterm delivery. Previous pregnancy losses were also foremost associated with increased risks of preterm PROM and preterm labor in deliveries before 32 weeks of gestation.

Among children born in Sweden between 1987 and 2002 we identified 1216 children diagnosed with autism and 6080 matched controls. Risks of autism associated with maternal and pregnancy characteristics (including maternal prenatal infections and other infectious related diagnoses) or neonatal complications were estimated using logistic regression models. To study the mediating effect of perinatal factors on the association between gestational age and autism we adjusted the models for maternal and pregnancy characteristics as well as neonatal complications in a stepwise manner. Infectious-related exposures were not associated with increased risk of autism. Compared with children born at term, the unadjusted odds ratios of autism (95 percent confidence intervals) among very (≤31 weeks) and moderately (32 to 36 weeks) preterm born children were 2.03 (1.13 to 3.64) and 1.52 (1.16 to 1.99), respectively. When we controlled for maternal and birth characteristics, corresponding risks were reduced to 1.48 (0.77 to 2.84) and 1.33 (0.98 to 1.81). After also controlling for neonatal complications, the risks of autism related to very and moderate preterm were further reduced. The reductions in risks of autism related to preterm birth were primarily attributed to preeclampsia, small-for-gestational age birth, congenital malformations, low Apgar score (0 to 6) at five minutes and neonatal brain injury.

LIST OF PUBLICATIONS

I. Buchmayer S, Sparen P, Cnattingius S Signs of infection in Pap smears and risk of adverse pregnancy outcome. *Paediatric and Perinatal Epidemiology* 2003, 17, 340-346.

II. Johansson S, Buchmayer S, Harlid S, Iliadou A, Sjöholm M, Grillner L, Norman M, Sparén P, Dillner J, Cnattingius S Infection with Parvovirus B19 and Herpes viruses in early pregnancy and risk of second trimester miscarriage or preterm birth. Reprod Toxicol. 2008 Oct 9. [Epub ahead of print] http://dx.doi.org/10.1016/j.reprotox.2008.09.012

III. Buchmayer S, Sparen P, Cnattingius S Previous pregnancy loss: Risks related to severity of preterm delivery. *American Journal of Obstetrics and Gynecology* 2004, 191, 1225-31.

IV. Buchmayer S, Johansson S, Johansson A, Hultman C, Sparen P, Cnattingius S Can the association between preterm birth and autism be explained by maternal or neonatal morbidity? *Submitted*

CONTENTS

LIST OF ABBREVIATIONS

AGA	Appropriate for gestational age
AOR	Adjusted odds ratio
ASD	Autism Spectrum Disorder
BMI	Body mass index
CI	Confidence Interval
CMV	Cytomegalovirus
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
ICD	International Classification of Diseases
IgM	Immunoglobulin M
IVH	Intraventricular hemorrhage
HHV-6	Human herpes virus 6
LGA	Large for gestational age
LMP	Last menstrual period
OR	Odds Ratio
PCR	Polymerase chain reaction
PROM	Premature ruptures of the membranes
RDS	Respiratory distress syndrome
SCB	Statistiska Centralbyrån (“Statistics Sweden”)
SGA	Small for gestational age
SLE	Systemic Lupus Etrythematosus
vs.	Versus
WHO	World Health Organization

INTRODUCTION

Infections during pregnancy have been associated with increased risks of both miscarriage and preterm delivery. Further, it has been hypothesized that exposure to prenatal or early life infections would affect fetal brain development and thereby increase the risk of neurodevelopmental aberrations in later life.

Although both miscarriages and preterm deliveries occurring during the second trimester have been found to be associated with intrauterine bacterial infections, most preventive strategies have been unsuccessful in preventing abortion or delaying preterm delivery. While much focus has been on bacterial infections, less is know about viral infections as a cause of adverse pregnancy outcome.

Miscarriages and preterm deliveries are often viewed as independent events. It is known that a woman with a previous miscarriage has an increased risk of miscarriage in a subsequent pregnancy. The same repetitive pattern is seen among preterm deliveries, in particular deliveries before the 32nd week of gestation. Since miscarriages and very preterm deliveries may share a common etiology in infections during pregnancy one may hypothesize that women with increased risk of infection would also be at risk of both miscarriage and preterm delivery.

Apart from causing miscarriages and/or preterm delivery it has been suggested that neurotropic infections during pregnancy or early life could have long-term effects on fetal brain development. Associations have been found between early life infections and later development of adult psychosis and schizophrenia. Autism is another neurodevelopmental disorder which has been hypothesized to be associated with prenatal infections. Further, children with autism are more often born preterm and it is not known whether the association is due to the short gestational length per se or other factors associated with both preterm birth and autism.

This thesis is comprised of four studies elucidating the associations between infections during pregnancy and risks of miscarriage, preterm delivery and later development of autism. The aims of the studies were to investigate the association between both specific infections and infection-related exposures during pregnancy on risks of miscarriage and preterm delivery. Further, we aimed to study the association between infections during pregnancy and risk of developing autism and whether prenatal infections or other factors could explain the association between preterm birth and risk of autism.

BACKGROUND

GENERAL INFECTIONS

An infection can be defined as the pathological state resulting from the invasion of the body by pathogenic microorganisms, such as bacteria, viruses, parasites or fungi. An infection can spread directly or indirectly, from one person to another. The symptoms observed during an infection may be caused by the infectious agent, or be a result of the host answer to the infection (the immune response). Although introduction of vaccination programs, antibiotics and improved living conditions have decreased mortality due to infections substantially, infections are still major causes of morbidity and mortality throughout the world[1, 2]. Further, new infections and associated conditions are continuously discovered.

Traditionally we associate infections with diseases like pneumonia, influenza or malaria. Recently though, infections have been hypothesized to be associated with a number of other conditions like atherosclerosis[3], leukemia[4], multiple sclerosis[5], type I diabetes[6], cervical cancer [7, 8] and adult psychoses[9-13]. The proposed mechanisms by which infections are associated with later disease vary. In atherosclerosis the increased risk is believed to be multifactorial but associated with inflammation in the vascular bed of the host[14]. In the case of cervical cancer, the HPV virus incorporates itself within the host DNA and thereby causes damages to DNA, leading to increased risks of cervical cancer[8].

The immune system

Very briefly, the immune system can be divided into: the innate or unspecific immune system and the acquired or adaptive immune system. The innate immune system always reacts in a similar fashion to an infectious agent. It includes both the barriers (skin, mucosa and cilia of the respiratory tract) and a number of different cells such as macrophages and neutrophils which together provide the first line of defense against a wide range of pathogens. These cells also play a role in activating the cells of the adaptive immune system, which consists of lymphocytes.

The adaptive or acquired immune system consists of specific defense mechanisms and a memory (immunity) for the previously encountered pathogens. Among others, the cells involved in this system are B- and T-lymphocytes, which are activated after contact with an antigen. B-cells produce antibodies acting against antigens in the blood and other extracellular spaces. T-cells are responsible for the cell-mediated immune response and can be divided into Th1 and Th2 cells where Th1 cells drive the type-1 pathway (cellular immunity) in which viruses and other intracellular pathogens are fought. The Th1 pathway also eliminates cancerous cells. Th2 cells drive the Th-2 pathway (humoral immunity) which stimulates B-cells to produce antibodies and fight extracellular organisms.

An imbalance between the two pathways can cause disease. Th1 is generally considered to be the more aggressive of the two pathways, and an overactive Th1

pathway has been associated with autoimmune diseases like arthritis, multiple sclerosis and psoriasis[15-17]. The Th2 pathway is seen as underlying allergy and related IgE based diseases[18-20].

Altering of the immune system during pregnancy

During pregnancy the woman's immune system is altered which most likely is an evolutionary strategy to prevent abortion of the fetus[21, 22]. It has long been believed that the Th2 pathway is up-regulated during pregnancy with a simultaneous down regulation of the Th1 pathway[23-26]. This is supported by the fact that diseases associated with Th1 activity, such as arthritis are usually less symptomatic during pregnancy, while Th2 dominated diseases, like SLE, tend to worsen during pregnancy[23]. Recently, this description of the immune system during pregnancy has been criticized as oversimplified. As an example, data indicates that Th1 activity is necessary for a successful implantation[24-26].

MISCARRIAGE AND PRETERM DELIVERY

Definitions

The duration of a normal singleton pregnancy has been estimated to around 40 weeks[27]. If the pregnancy is ended after the beginning of the 37th weeks and before the ending of the 41st week it is considered a term delivery. Pregnancy loss or delivery before this time is classified as a miscarriage, a stillbirth or a preterm delivery.

According to the World Health Organization (WHO), a miscarriage is a fetal death in early pregnancy. At what gestational age a miscarriage becomes a stillbirth for reporting purposes depends on each country's policies. In Sweden the legal definition of miscarriage was until recently an involuntary pregnancy loss before 28 weeks of a fetus without vital signs, while an infant born without vital signs after the 28th week of gestation is classified as stillborn. As the survival of premature infants born before 28 weeks has increased, the upper cut-off limit for miscarriage has been reduced and today health staff working at delivery clinics report fetuses born, without vital signs, between 22 and 28 weeks as stillborn.

Miscarriages are often subdivided into first trimester miscarriages (before 14 weeks gestation) and second trimester miscarriages (14-22 weeks gestation). They can also be divided into sub-clinical and clinical miscarriages. A sub-clinical miscarriage occurs before the woman realized that she is pregnant, while a clinical miscarriage is one that is clinically detected by the woman.

Preterm delivery is defined as a delivery before 37 completed gestational weeks, with very preterm deliveries being before 32 weeks, while deliveries between 32 and 36 weeks of gestation are defined as moderately preterm. Sometimes very preterm delivery is further divided into extremely preterm, i.e., deliveries before 28 weeks gestation. In this thesis we use the terms extremely, very and moderately preterm delivery.

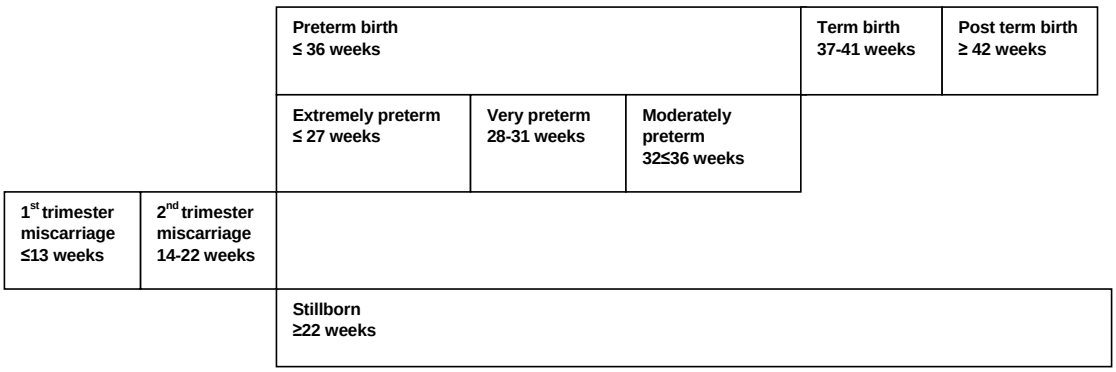


Figure 2. Definition of miscarriages, stillbirths and preterm births by gestational weeks.

Incidence rates

Measuring incidence rate of miscarriage is not easy since sub-clinical miscarriages are by definition not recognized by the woman. However, all evidence suggests that these miscarriages are by far the most common[28-30]. In a study including 221 women who attempted to become pregnant, the rate of miscarriage in early pregnancy was estimated measuring HCG-levels in urine[28]. They found that in total 31% of pregnancies were spontaneously lost, of which 22% of the pregnancies were lost before they were clinically recognized[28]. The incidence rate of second trimester miscarriages has been reported to be approximately 1-2% [31].

The incidence of preterm delivery varies worldwide. In Sweden, approximately 6-7 % of all deliveries are preterm, with 1-2% occurring before the 32nd week of gestation[32]. The rate of preterm birth has remained approximately the same over the last decades as have the percentage of preterm born children with handicaps. In contrast, the mortality among very preterm infants has decreased substantially which has led to an increase in the absolute number of preterm born children with handicaps. These very preterm born infants constitute the main problem in neonatal care today, since the mortality and morbidity is by far the greatest in this group[33].

Risk factors

The causes of miscarriage and preterm delivery are not fully understood and there are a number of different risk factors for miscarriage and preterm delivery, working through different mechanisms at different gestational ages. In early pregnancy, genetic abnormalities of the fetus are believed to be the dominating cause[34]. Maternal bacterial infections during pregnancy have been associated primarily with increased risks of second trimester miscarriages and very preterm deliveries[35].

Genetic factors

Genetic and other chromosomal abnormalities are associated with increased risks of both miscarriage and preterm delivery[34, 36]. The risk of pregnancy loss in association with genetic abnormalities is foremost seen in early pregnancy, because many chromosomal abnormalities may be lethal or in other ways significantly reduce the viability of the fetus[37]. Presence of genetic abnormalities is also, as described below, closely related to increasing maternal age as a main risk factor for both miscarriage and preterm delivery.

Environmental and nutrient factors

A number of environmental factors have been seen to influence the risk of miscarriage and preterm delivery. Folic acid deficiency has been found to increase risk of early spontaneous abortions, possibly by interacting with the rapid DNA synthesis in early pregnancy[38]. Exposure to radiation or drugs during pregnancy is associated with increased risk of both miscarriage and preterm delivery. For example, maternal smoking during pregnancy increases the risks of both miscarriage[39] and preterm delivery[40], but also environmental exposure to passive smoking has been found to increase the risk of miscarriage[41]. By which mechanism this would work is not known, but it is known that nicotine alters the immune system and it is possible that smoking women are at increased risks of infection during pregnancy[42] which could increase their risk of miscarriage and preterm delivery[40].

Maternal factors

Increasing maternal age is one of the main risk factors for both miscarriage and preterm delivery[31, 43, 44]. This is most likely explained by a lowered "quality" of the female egg which increases the rate of meiotic errors resulting in genetic abnormalities. Increasing maternal age has been associated with increased risks of almost all trisomies [45], but the increased risk of miscarriage also applies to fetuses with normal karyotype[31]. Also younger women (below the age of 20) are reported to have increased risks of preterm delivery[46-50]. This is believed to be related foremost to lower socioeconomic status and less attendance to maternal care programs, leading to both increasing stress, lowered compliance with general health recommendations, resulting in complications during pregnancy not being detected[50, 51]. It has also been hypothesized that teenage mothers would be at increased risk of infections during pregnancy, which in turn could increase their risk of miscarriage or preterm delivery.

A number of other maternal related factors, such as some chronic diseases (SLE, hyperthyroidism, inflammatory bowel disease) and maternal stress[52-55] have also been found to increase the risk of preterm delivery and miscarriage[56]. Multiple pregnancies are also associated with increased risks of both miscarriage and preterm delivery.

Previous miscarriage or preterm delivery

The foremost predictive factor for both miscarriage and preterm delivery is a previous miscarriage or preterm delivery[57-59], especially a very preterm delivery. Different explanatory models have been suggested: Women with any of the above mentioned risk factors in the first pregnancy would be at increased risk in the second pregnancy as well

if the risk factor is still there [57, 59]. However, even when these risk factors are controlled for, there is still an increase in risk [57]. Since there is a strong genetic component in both miscarriages and preterm deliveries, there is also most likely a high heritability for the events. It has also been suggested that part of the repetitive pattern could be due to infections; some women may carry chronic infections or have higher general susceptibility for infections which could make them more prone to have a miscarriage or a spontaneous early onset of delivery[60, 61]. Further, there are also studies indicating that inflammatory responses may be affected by genetic factors[62-67].

Infection as a risk factor for miscarriage and preterm delivery

As early as in the 1950s it was suggested that uterine infections may be associated with preterm delivery[68]. In the 1970s, intrauterine infection of women without previous rupture of the membranes was first linked to preterm delivery[69, 70]. Since then a vast amount of research has been conducted within the field of infections during pregnancy and later risk of adverse pregnancy outcomes[61, 71, 72].

Most conducted studies have found associations primarily between infections during pregnancy and increased risk of second trimester miscarriage[73] or delivery before 32 weeks [74], while the association is not as clear with moderately preterm deliveries (after 32 gestational weeks). Why this pattern is seen has not been fully elucidated, and it is also not clear when the woman is infected. Most infections associated with preterm delivery and miscarriages are asymptomatic and may remain undetected until they initiate miscarriage or spontaneous preterm delivery[71]. Some studies have suggested that the woman may be infected in early pregnancy or even before she becomes pregnant[75-78]. In mid-pregnancy the endometrial cavity is sealed, and one hypothesis has been that if the infection is not cleared within four to eight weeks after this, the woman may develop symptoms resulting in miscarriage or preterm delivery[71]. It has also been suggested that the association primarily found with late miscarriages and very preterm deliveries may be a result of the immune response of the fetus; with an older fetus being able to initiate a more mature immune response including the generation of cytokines and hormones necessary to initiate preterm delivery.

Routes of infection

There are a number of different routes by which an infection may reach the intra-amniotic cavity; a blood-borne infection may disseminate through the placenta, an infection of the abdominal cavity could spread via the fallopian tubes, medical interventions such as amniocentesis may also introduce infections, but most commonly the infection is likely to reach the uterus through the vagina and cervix.

Bacterial infections from the vagina are believed to ascend through the cervix into the choriodecidual space. Some infections may then cross the intact membranes into the amniotic fluid and possibly also infect the foetus (Figure 3). This route of infection has been supported by studies which show that women with positive membrane cultures also show presence of the same organisms in the amniotic fluid[79]. Further, these are often organisms with vaginal origin. Some studies have also found the same organism in foetuses which were preterm born.

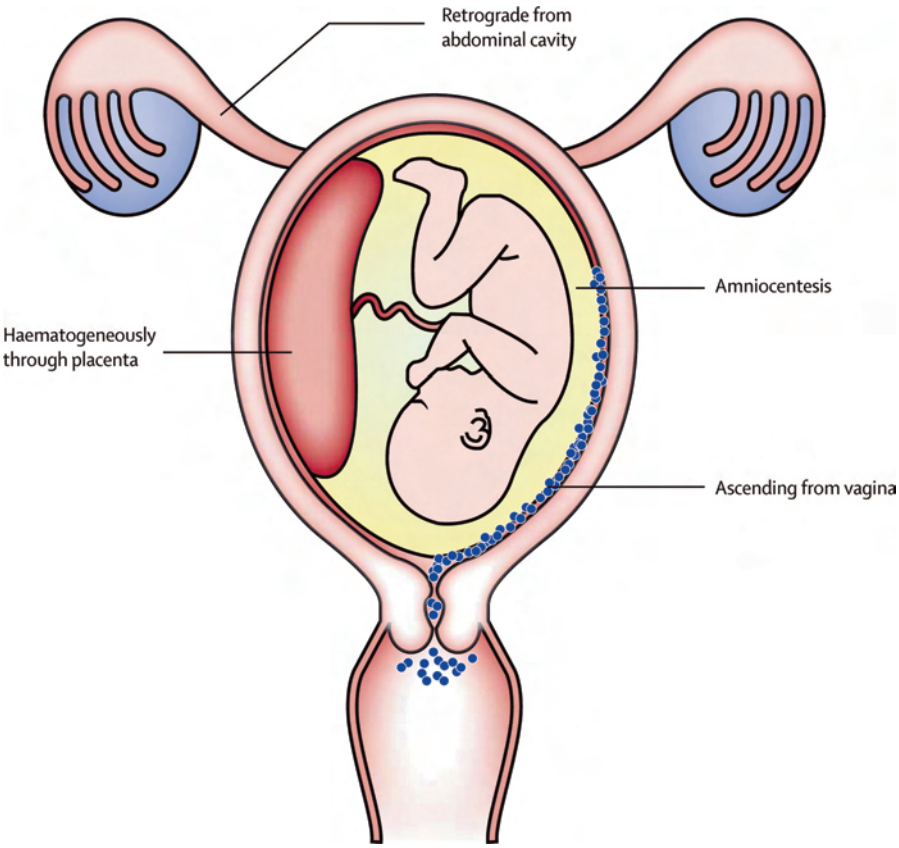


Figure 3. Routes by which infections may reach the uterus. (From Goldenberg, Culhane, Iams and Romero. Epidemiology and causes of preterm birth. Lancet 2008;371:75-84.[80], Reproduced with permission).

Mechanisms of infection

The mechanism by which bacterial infections cause miscarriage or preterm delivery are believed to be related to inflammation[71, 81]. Bacterial infections release toxins which activate cytokine production, including interleukin-6 (IL-6), tumour necrosis factor α (TNFα) and granulocyte colony-stimulating factors (G-CSF). Cytokine production then stimulates neutrophil infiltration and prostaglandin synthesis leading to increased levels of metalloproteases which in turn causes cervical ripening, weakening and rupture of the chorioamniotic membranes. Prostaglandins also stimulate contractions of the myometrium and thereby cause preterm labour. This inflammatory response may then culminate in spontaneous abortion or preterm delivery (Figure 3).

The fetus has a natural barrier towards infection consisting of the cervix, the chorioamniotic membranes and the placenta, but fetuses which get infected may activate an inflammatory response leading to preterm labor. Infections of the fetus have been shown to cause an increase in corticotrophin releasing hormone (CRH) leading to increased levels of cortisol which stimulates prostaglandin synthesis[82] (Figure 3).

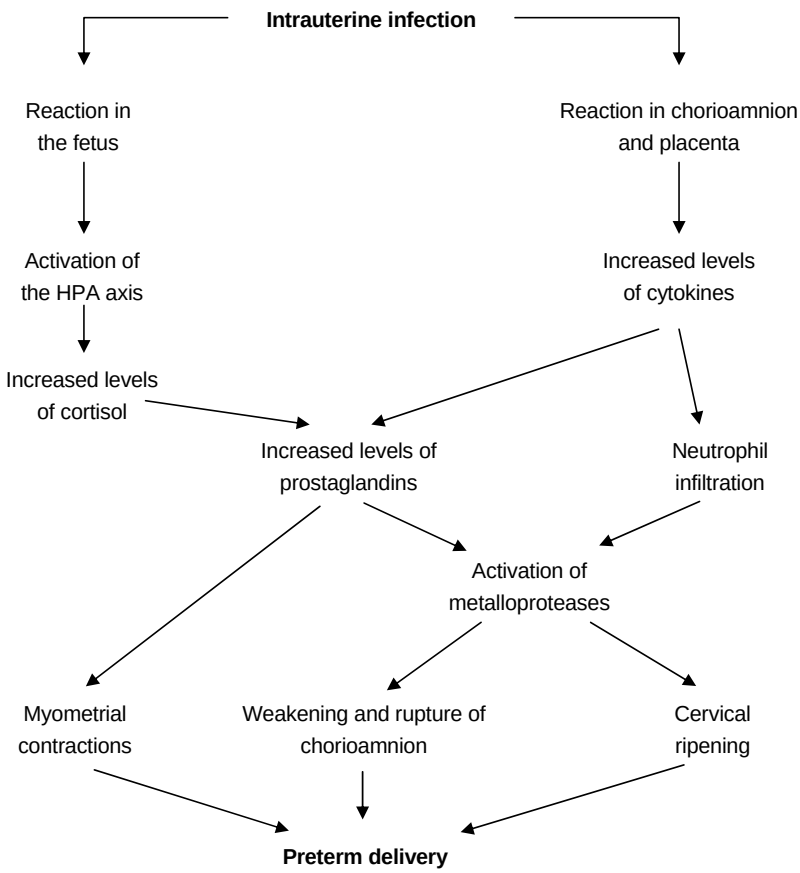


Figure 4. Potential mechanism by which intrauterine infection could cause miscarriage or preterm delivery. (From Goldenberg, Hauth, Andrews. Mechanisms of disease: intrauterine infection and preterm delivery. New Eng J Med 2000;342(20) [71], Reproduced with permission).

Detection

As mentioned earlier, most infections associated with preterm delivery and miscarriage are not related to any particular clinical symptoms such as vaginal discharge, fever or a tender uterus. The infections are therefore often not recognized by the woman or her physician until she develops symptoms such as a vaginal bleeding or a premature rupture of the membranes or premature labour. As a consequence much research has focused on detecting women at risk, and finding early markers of infection.

Treatment

With few exceptions, treatments of infections during pregnancy have so far not proven to delay or decrease the risk of preterm delivery or miscarriage. If this is because the patients are administered drugs which are not effective against the infection, or because the treatment is given too late is not known.

During the 1970s, women with asymptomatic urinary tract infection during pregnancy were routinely given tetracycline since this had shown to reduce rates of preterm delivery[83]. This treatment was later abandoned, primarily due to the negative effects on foetal dental and bone development. Recently, new studies have shown that treatment of both symptomatic and asymptomatic urinary tract infection with other drugs may reduce the risk of not only pyelonefritis but also preterm delivery[84]. In fact, treatment of asymptomatic urinary tract infection and Bacterial vaginosis (see below) are some of the few interventions which have been suggested to prevent preterm birth.

Bacterial vaginosis

Bacterial vaginosis, a normally asymptomatic condition which may be present in as many as 20% of all pregnancies, has consistently been associated with increased risks of preterm birth and miscarriage[35, 85-87]. It is defined as an altering of the vaginal flora rather than infection with a single micro-organism[88, 89]. Most commonly Bacterial vaginosis is diagnosed by gram staining, but some studies have concluded that the presence of coccobacilli on Pap smear is a superior method in detection of bacterial vaginosis[90-92]. Treatment of Bacterial vaginosis has been shown to reduce risks of preterm delivery, especially in women with previous preterm deliveries[93, 94], but there are also studies indicating no benefit from treatment[88].

Other organisms

A number of other bacteria have also been cultured from amniotic fluid and chorioamnionitic membranes in preterm deliveries; such as *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis* and Peptostreptococci[95-97]. These are all vaginal organisms with a generally low virulence. Escherichia coli, Chlamydia and Gonorrhea are rarely found in the uterus before rupture of the membranes[97]. Group B streptococci infection, which is associated with increased risks of neonatal sepsis, has not been shown to increase the risk of preterm delivery.

Infections localized to organ systems such as pneumonia and other systemic infections have also been found to predispose to preterm delivery[98]. There are studies indicating that periodontal infections may more than double the risk of preterm delivery, but more studies within the field are needed[99, 100].

Viral infections

While much focus has been on bacteria, less is known about the role of viral infections. The only larger epidemiological study to date suggested that *Parvovirus B19* may be associated with an increased risk of late spontaneous abortion and stillbirth[101]. The prevalence of IgM seropositivity for *Parvovirus B19* among women with such

pregnancy complications was 13 percent as compared to 1.5 percent in the remaining pregnant population[101]. Smaller clinical studies and case-series also report that viral infections may increase the risk of preterm delivery. Herpes viruses have also been reported as a risk factor of miscarriage and preterm delivery. Miscarriage was reported to be more common among pregnant women positive for HHV-6 compared to HHV-6 negative women[102, 103]. Levels of antibodies against *Cytomegalovirus* was found to be higher in women with early onset preeclampsia and preterm delivery, compared to women with normal pregnancies ending at term[104]. *Cytomegalovirus* detected in dried neonatal blood spots sampled after birth, was also more common in infants born preterm than term[105].

AUTISM

Autism is a developmental disorder which causes substantial suffering for both the individual affected and his or her family. The symptoms usually occur in early childhood and approximately 75% of the children affected are boys. Autism is not a single disease but rather a syndrome with clinical features which may vary greatly, but all include a triad of symptoms related to communication, social skills and repetitive behaviors[106, 107]. A severely affected child may be mentally retarded without a language and preoccupied with stereotypical behavior; while a mildly affected child can be highly functioning, have a well developed language but lack in social competence and be obsessed with certain rituals and routines setting him or her apart as an “odd” personality[107]. The term Autism Spectrum Disorder (ASD) has been introduced to cover this wide spectrum of symptoms.

The prevalence of autism varies widely between studies, from studies indicating a prevalence of 5 per 10000 born children to almost 65 per 10000[108-110]. This is most likely a reflection of the wide spectrum of the disorder, different inclusion criteria between studies and also a development of diagnostic procedures. There are a number of studies indicating an increase in autism during the last decades. It is not clear whether this is a true increase or merely a result of more children receiving the diagnosis due to an increased awareness of the syndrome[111].

Risk factors

The etiology of autism is most likely multifactorial, especially considering the phenotypic variation and genetic complexity within the syndrome[107]. A number of factors are associated with an increase in risk of developing autism and much research focuses on further exploring these and other risk factors. Early identification of individuals at risk is essential since treatment and training has shown to have a positive effect on symptom development.

Genetic factors

Twin studies show a much greater concordance of autism in monozygotic than in dizygotic twins suggesting a substantial genetic component in autism[112]. The recurrence risk for siblings is also much higher than the general population risk, but much lower

than in single genetic traits, providing further evidence for a complex but strong genetic component. Also, the relatives of children with autism have shown a higher degree of milder phenotypes of the disorder. This has led to the conclusion that autism is influenced by complex genetic components, one hypothesis being that a number of genes interact with each other in the development of autism.

Parental factors

Increasing maternal age as well as increasing paternal age has been found to increase the risk of receiving a child who develops autism[113-115]. This could be explained by an increasing rate of mutations in the eggs and sperm with increasing maternal and paternal age. Also, the increased risk of autism associated with increasing maternal age could be due to pregnancy complications associated with high maternal age.

Immigration status of the mother has repeatedly been associated with increased risks of autism[116-118]. It has been speculated whether this could be a consequence of the migrating mother being exposed to a different spectrum of infections or changes in diet which could induce immune responses or metabolic disorders[118]. Another hypothesis is that men displaying autism-like symptoms are more likely to marry foreign women who are not as likely to detect social and/or language difficulties[117].

Other factors which have been found to be associated with increased risks of autism are maternal smoking[118], and a maternal history of psychoses[114].

Perinatal factors

Infants who later develop autism have an increased frequency of pre- and perinatal complications compared with unaffected children[114, 116, 118, 119]. It has therefore been suggested that autism may be a disease with a prenatal origin where early environmental insults affect fetal brain development and predispose to autism.

Preterm birth has repeatedly been associated with an increased risk of autism[114, 116, 118-121]. It is not known whether this association reflects a general immaturity of the brain of the preterm born infant or whether it is due to other factors also associated with preterm birth. A number of studies have also shown that both low birth weight and being born small for gestational age are risk factors which predisposes to autism[110, 111, 113-115]. Further, congenital malformations significantly increase the risk of autism[118, 120].

One commonly discussed hypothesis has been that infections of the central nervous system during a critical point in pregnancy increase the risk of developing autism and other neuropsychiatric disorders[122, 123]. Prenatal infections and inflammation have been associated with increased risk of cerebral palsy in the offspring[105, 124-126]. The hypothesis is also supported by the association between infections of the central nervous system in early life and increased risks of schizophrenia[127, 128]. Chess showed that 8-13% of children born during the 1964 rubella pandemic developed autism along with other birth defects also associated with congenital rubella syndrome[129]. Other infectious agents may also possibly be related to autism[123, 130-132]. A number of studies have also shown a deviation in the immune response of autistic children indicating that inflammation may play a role in the etiology of autism[133-135].

In the late 1990s, much media attention was given to a study of inflammatory bowel disease in children with autistic disorders[136]. The study pointed at a possible connection between Measles, Mumps and Rubella vaccination (MMR) and the development of autism. One interpretation made from the study was that the increase in autism could be associated with the introduction of MMR vaccination. Although a number of later studies found no support for a causal association between MMR vaccination and autism and the original authors also published a withdrawal of the original interpretation, the results from this first study probably contributed to a substantial decrease in MMR vaccination of children in the early 2000s[137-139].

Children who later develop autism are also more frequently transferred to the neonatal intensive care unit after birth[120] and are reported to have an increased frequency of neonatal complications including a low Apgar score[114, 115, 118], respiratory distress[140], seizures[140], and other neonatal brain insults[141].

AIMS

The aim of this thesis was to investigate whether:

- Infectious signs in Pap smears increase the risk of preterm delivery
- Specific viral infections during pregnancy increase the risk of miscarriage or preterm delivery
- Miscarriage increases the risk of preterm delivery
- Prenatal infections increase the risk of developing autism and whether this could explain the association between preterm delivery and autism.

MATERIAL AND METHODS

SETTINGS

All of the studies included in this thesis have been conducted in Sweden, a country with exceptional opportunities for epidemiological studies. This is mainly attributed to the existence of nationwide registers, the systematic use of a unique personal registration number and a health care system which is accessible for all citizens.

The personal registration number

Since 1947 and onwards all Swedish residents are assigned a unique ten-digit number at birth or immigration. This ten-digit number consists of six digits giving birth year, month and day, which combined with four more digits, creates a unique identifier for each citizen. Through the use of this personal registration number it is possible to link individual information from different data sources.

DATA SOURCES

Medical Birth Register

Practically all births in Sweden since 1973 are registered in the Medical Birth Register, held by the National Board of Health and Welfare[142]. Starting with the first antenatal visit, information is prospectively collected, including maternal demographic data, information on reproductive history and complications during pregnancy, delivery and the neonatal period. The register contains among other things information on birth weight, infant sex, and gestational age at delivery. All births and perinatal deaths are validated each year against the Register of Population and Population Changes, held by Statistics Sweden. Further, all information is individually linked to the women's unique personal registration number. Information from the Birth register was used in studies I-IV.

The Hospital Discharge Register (the Inpatient Register)

Everyone who is admitted to in-patient hospital care in Sweden is registered in the Hospital Discharge Register, held by the National Board of Health and Welfare, which has a nationwide coverage since 1987. This register contains information on the patient's diagnoses, admission and emission dates, and the patient's unique personal registration number. The diagnoses are coded according to the recommendations by the International Classification of Diseases (ICD); with the eight revision (ICD-8) used before 1987, the ninth revision (ICD-9) used from 1987 through 1996, and the tenth revision (ICD-10) from 1997 and onwards. The Hospital Discharge Register, which is also called the Inpatient Register, was used in studies II-IV.

The Pap Smear Screening Register

The National Board of Health and Welfare recommends that all women in Sweden are called for regular Pap smear screening. This program, with the aim of early detection of

cervical cancer, was started in the counties of Uppsala and Gävleborg in 1969, and has since then spread throughout the country. Today all Swedish women should be called and have a Pap smear taken every third (women aged 23-50) or fifth year (women aged 51-60). Information on all Pap smears are registered in the Pap Smear Screening Register, including information on cytological findings, test dates, infectious signs in Pap smear, and the women's unique personal registration numbers. The Pap Smear Screening Register was used in study I.

The Register for Stored Blood Samples

Since 1975, the Swedish maternity care program includes serological screening for Rubella immunity in early pregnancy. Sera from the Rubella screening are stored according to requirements issued by the National Board of Health and Welfare[143]. In the county of Stockholm 75,037 blood samples from the Rubella screening were taken and stored at the Virology Laboratory of Karolinska University Hospital (Solna), Sweden between the years 1990 to 2002. The Register for Stored Blood Samples was used in study II.

Medical records

Data obtained from registers is invaluable, particularly by giving us information on a vast amount of individuals, data which would be both logistically and financially impossible to obtain in other ways. Also register data has the advantage of being prospectively collected, thereby minimizing selection bias. But register data is not always enough; therefore in study II we also collected data from medical records of individuals with miscarriages and preterm deliveries.

STUDY DESIGN

Paper I

By using the mothers' unique national registration numbers it was possible to link the Regional Pap Smear Screening Register to the Medical Birth Register and construct a database including all women in the counties of Uppsala and Gävleborg between 1973 and 2000, who had taken a Pap smear during pregnancy (n= 60 755).

Infectious signs in Pap smears consisted of four different parameters: Inflammation, Trichomonas vaginalis, Coccobaccilli or Fungus. Presence or absence of inflammation was determined by the amount of leukocytes present in the smear. Abundant amounts of leukocytes were considered a positive result for inflammation, while moderate amounts of leukocytes could be present without a positive result. The Pap smears were also visually analyzed by microscope for the presence or absence of Trichomonas vaginalis, Coccobaccilli or Fungus.

The following outcomes were used: antepartal death, preterm delivery ($\leq$36 weeks) and small-for-gestational-age (SGA) delivery. An antepartal death was defined as a fetal death before labor occurring at 28 completed weeks or later. Preterm delivery was stratified into very preterm delivery (below 32 completed weeks) and moderately preterm (32-36 completed weeks). SGA was defined as birth weight more than 2 standard deviations below the mean birth weight for gestational age, according to the Swedish fetal growth curve in common use[144].

Gestational age was estimated by second trimester ultrasound where available, otherwise the last menstrual period was used. In Sweden, ultrasound was introduced as a tool to determine gestational age in 1973, and since 1990 (which includes one-third of our sample) it has been used in 95% of all pregnancies[145].

Statistical Analysis

The presence of infectious signs in Pap smears was analyzed, using $\chi 2$ to test for differences between categories. Risk of antepartal mortality, very preterm delivery, moderate preterm delivery, and delivery of an SGA infant, with respect to presence or absence of infectious signs were analyzed in logistic regression models. Risks of preterm and SGA deliveries were also analyzed in a subgroup, only including women with Pap smears taken within 4 weeks before delivery. In the analyses of preterm and SGA deliveries, we only included live births.

All analyses were adjusted for maternal age, parity, previous preterm delivery, county and decade of test. We also adjusted for calendar year in which the test was performed, in an attempt to control for differences in measurement of gestational length.

Paper II

This case-control study was based on pregnant women residing in Stockholm County during the years 1990-2002 who had a blood sample taken in early pregnancy. The Swedish maternity care program includes serological screening for Rubella immunity in early pregnancy. Sera from the Rubella screening are stored according to requirements issued by the National Board of Health and Welfare[143]. On average, blood samples included in this study had been sampled at 11 completed gestational weeks, and 90 percent of samples had been collected between 7 and 18 weeks of gestation.

To obtain information about pregnancy outcome, the Register for Stored Blood Samples at Karolinska University Hospital (Solna) was linked to the Swedish Hospital Discharge Register and the Swedish Medical Birth Register. After register linkage, cases and controls were selected. Cases were defined as women with pregnancies with a gestational length from 14 to 31 completed weeks, and included second trimester miscarriages (pregnancy loss from 14 through 22 completed weeks) and very preterm deliveries (delivery of live born singleton infants from 23 through 31 completed weeks). We randomly selected 500 controls with singleton live term (>37 completed weeks) births.

We aimed at retrieving a complete set of serum samples for virus analyses from all potential cases and controls. However, serum samples collected from 1990 through 1992 had, after the study was initiated and without our knowledge, been cleared from the Virology laboratory. The reason for this was that the National Board of Health and Welfare mandates storage of samples only for 10 years[143]. Consequently, only 235 cases with second trimester miscarriages, 269 cases with very preterm live singleton births and 301 controls with live singleton term births (>37 weeks) had serum samples taken between 1993 and 2002 which could be analyzed.

Women were regarded as exposed to viremia when they had early pregnancy blood samples positive for viral RNA (PCR technique) from the following viruses: Human Parvovirus B19, Varicella Zoster, Cytomegalovirus, Epstein Barr virus, Human Herpes virus 6, Human herpes virus 7 or Human herpes virus 8. The laboratory was blinded to the case and control status of the samples.

Statistical Analysis

The effect of viremia on adverse pregnancy outcome was estimated by logistic regression models. Maternal age and number of previous births were a priori considered as potential confounders and were included in adjusted models.

Differences in gestational age, birth weight and etiology of very preterm delivery, between virus positive and virus negative preterm cases were assessed by Wilcoxon-Mann-Whitney test or $\chi 2$-test.

Paper III

In study III we used the national registration numbers to construct a database which, in addition to the birth information from the Medical Birth Register, also included information about the women's previous pregnancy losses, obtained from the Hospital Discharge Register. This study was restricted to primiparous women with live singleton births from 1987 through 2000 (n=601 883).

The number and type of previous pregnancy loss was registered for each woman. Pregnancy losses were classified into tree different types: spontaneous abortion, missed abortion and ectopic pregnancy. The number of miscarriages was categorized as follows: no previous spontaneous abortion, one spontaneous abortion, or two or more spontaneous abortions. Information about previous missed abortion and ectopic pregnancy was categorized as yes or no.

Preterm delivery was stratified into extremely preterm delivery (22-27 completed gestational weeks), very preterm delivery (28-31 weeks) and moderately preterm delivery (32-36 weeks). From the Medical Birth Register we also retrieved information on diagnosis of women with preterm deliveries before 37 weeks of gestation. Diagnoses of deliveries before 32 weeks and 32-36 weeks were hierarchically stratified into three groups as follows: preterm premature ruptures of the membranes (preterm PROM), preterm labor but no preterm PROM, and other reasons for preterm delivery.

Gestational age was estimated by second trimester ultrasound when available; otherwise the last menstrual period was used.

Statistical Analysis

The risk of extremely preterm delivery, very preterm delivery and moderately preterm delivery associated with previous pregnancy loss was estimated in logistic regression models. The risk of preterm delivery was also assessed with respect to number of pregnancy losses and interpregnancy interval.

To investigate causes of preterm delivery, we also estimated the risks of preterm PROM, preterm labor and other reasons for preterm delivery both before 32 weeks and between 32 and 36 weeks of gestation.

All analyses were first made unadjusted and then adjusted for maternal age, relationship with the father, smoking habits, mothers' country of birth, and birth year.

Paper IV

We designed a case-control study, nested within the population-based cohort of all live born infants in Sweden between 1987 through 2002 recorded in the Swedish Medical Birth Register.

By using the national registration numbers, we cross-linked this cohort with the Hospital Discharge Register and identified 1216 children who had been diagnosed with autism between the years of 1987 and 2005 and before the age of 10 years (ICD-9 code 299 and ICD-10 code F84). Of these 1216 cases, 868 had infantile autism (ICD-9 code 299A and ICD-10 code F840) and 348 had other autistic disorders (ICD-9 code 299B-X and ICD-10 code F841-9). For each case, we randomly selected five controls, individually matched by sex, birth year, and birth hospital. All controls were alive and not diagnosed with autism at the time of diagnosis for the case.

Information regarding previous pregnancy losses (spontaneous abortions or missed abortions) was retrieved from the Hospital Discharge Register. From the Medical Birth Register, we retrieved information regarding the following maternal characteristics: maternal age at delivery, smoking, maternal country of birth, if the mother cohabited with the father or not and maternal schizophrenia. Further, data on maternal infections during pregnancy and the infectious related diagnoses of previous preterm delivery, previous miscarriage, involuntary childless years or season of birth were collected. Information on maternal diabetes, preeclampsia, and gestational hypertension was also included.

Gestational age at delivery was divided into very preterm, moderately preterm, term and post-term, occurring at $\leq$31, 32-36, 37-41, and $\geq$42 completed gestational weeks, respectively. Gestational age was estimated by second trimester ultrasound when available; otherwise the last menstrual period was used.

Further, infants were categorized as being small-for-gestational-age (SGA), appropriate-for-gestational-age (AGA), or large-for-gestational-age (LGA). SGA and LGA were defined as birthweight more than 2 standard deviations below (SGA) or above (LGA) the mean birthweight for gestational age, according to the Swedish fetal growth curve in common use[144]. Apgar score at five minutes and information on multiple births were also registered. Further, we included information about the following neonatal diagnoses: congenital malformations, intracranial bleeding, cerebral edema or seizures, delivery associated head or neck injuries, neonatal infections, hypoglycemia, respiratory distress, and neonatal jaundice.

Statistical Analysis

The data was analyzed using conditional logistic regression, conditioning on the individual matching strata.

The models were adjusted for maternal age at delivery, smoking, maternal country of birth, if the mother cohabited with the father or not and maternal schizophrenia.

To study the effects of perinatal factors on the association between gestational age and autism, we further adjusted the models for pregnancy characteristics, birth characteristics and neonatal complications in a stepwise manner.

Interaction analyses were performed to investigate whether associations between neonatal complications and autism were different in preterm (≤36 weeks) and term (≥37 weeks) born children. The interactions were formally tested using likelihood ratio tests from which p-values are reported.

Ethical considerations

All studies included in the thesis were approved by the research ethics committee of the Karolinska Institutet (Study I, Dnr 98-002 + Dnr 02-556; Study II, Dnr 02-084; Study III, Dnr 02-008; Study IV, Dnr 2008/281-31/4).

RESULTS

PAPER I

Generally, presence of inflammation, Coccobaccilli or fungus in Pap smears taken during pregnancy did not imply an increased risk of preterm delivery. Trichomonas vaginalis was associated with an increased risk of moderate and possibly also very preterm delivery, while small-for-gestational-age infants were more often delivered by mothers with presence of Coccobaccilli. When studying antepartal stillbirth, no increased risk was found among women with infectious signs in Pap smears taken during pregnancy (results are presented in Paper 1, Table 3).

In a supplementary analysis, we only included women with Pap smears taken within four weeks before delivery (Table 1). We found that women who tested positive for Coccobaccilli had a more than four-fold increased risk of very preterm delivery, compared with women with Pap smears negative for Coccobaccilli.

Table 1. Adjusted[a] odds ratios (OR) and 95% confidence intervals (CI) for the association between infectious signs in Pap smears taken within 4 weeks before birth and risk of preterm or small for gestational age (SGA) birth.

		Very preterm delivery[b] (23 ≤ 31 weeks)		Moderately preterm delivery[c] (32 ≤ 36 weeks)		Small for gestational age[d]	
Test result	Total N	N (%)	OR (95% CI)[a]	N (%)	OR (95% CI)[a]	N (%)	OR (95% CI)[a]
Inflammation							
Positive	93	3 (3.2)	0.5 (0.1-2.2)	13 (14.0)	1.6 (0.7-3.4)	6 (6.5)	1.8 (0.6-5.1)
Negative	340	15 (4.4)	1.0	32 (9.4)	1.0	15 (4.4)	1.0
Coccibacillus							
Positive	49	5 (10.2)	4.7 (1.4-16.1)	6 (12.2)	1.3 (0.4-3.7)	1 (2.0)	0.3 (0.0-2.1)
Negative	384	13 (3.4)	1.0	39 (10.2)	1.0	20 (5.2)	1.0
Trichomonas							
Positive	12	0 0		1 (8.3)	0.6 (0.0-3.2)	0 (0)	0.0
Negative	421	18 (4.3)	1.0	44 10.5)	1.0	21 (5.0)	1.0
Fungus							
Positive	31	2 (6.5)	2.0 (0.4-10.0)	4 (12.9)	1.1 (0.3-4.1)	1 (3.2)	0.5 (0.1-4.4)
Negative	402	16 (4.0)	1.0	41 (10.2)	1.0	20 (4.9)	1.0

[a] Adjusted for maternal age, parity, decade of test, and county of test.
[b] Reference group is women negative for infectious signs, gestational age ≥32 weeks.
[c] Reference group is women negative for infectious signs, gestational age ≥37 weeks.
[d] Reference group is non-SGA births.

When we also analyzed tests taken between five and eight weeks before delivery, the presence of Coccobaccilli did not significantly increase the risk of very preterm birth (data not shown). The presence of inflammation, Trichomonas or fungus in Pap smears taken within four weeks before delivery was not associated with increased risks of preterm or small for gestational age delivery (Table 1). Due to low numbers of antepartal deaths it was not possible to restrict the analyses of antepartal deaths to only include Pap smears taken within 4 or 8 weeks before delivery.

PAPER II

In early pregnancy, infection with Parvovirus or Herpes virus was more often detected in blood samples from cases compared to controls. Viremia was found in 21 cases, but only in 5 controls, and was associated with a more than two-fold risk of second trimester miscarriage or very preterm birth, of borderline statistical significance. Of the 26 virus positive samples, Parvovirus B19 was detected in samples from 14 cases and 3 controls, and seemed to be associated with an almost threefold increase in risk. Human Herpes virus 6 (HHV6) was detected in serum samples from 6 cases and 2 controls. Cytomegalovirus (CMV) was only found in one sample (from a woman with very preterm delivery). Analyses stratified for virus type indicate that Parvovirus B19 may be associated with a higher risk compared to HHV6 (results are available in Paper II, Table 2).

Next, cases were divided into second trimester miscarriages and very preterm births and risks for these outcomes were calculated separately (Table 2). Viremia, regardless of virus type, seemed to increase the risk of both outcomes, although none of the associations were significant. Stratified analyses by virus type showed that risk estimates were consistently elevated for Parvovirus B19, whereas HHV6 was, if anything, only associated with an increased risk for second trimester miscarriage.

Finally, associations between viremia and characteristics of very preterm births were explored. We hypothesized that early pregnancy viremia would in particular increase the risk of extremely preterm birth (delivery before 28 weeks) with spontaneous onset of labour. Hence, we investigated whether the group of very preterm infants to virus positive mothers were different from the group of very preterm infants to virus negative mothers. However, aetiologies of very preterm delivery were similarly distributed in the two groups, as were gestational age and birthweight.

TABLE 2: Prevalence of viral infections and odds ratios (OR) with 95% confidence interval (CI) of miscarriage or very preterm birth.

	Term birth (≥ 37 weeks)		2nd trimester miscarriage (14-22 weeks)						Very preterm birth (23-31 weeks)					
					OR (95% CI)						OR (95% CI)			
Test results	**N**	**(%)**	**N**	**(%)**	**Crude**		**Adjusted[a]**		**N**	**(%)**	**Crude**		**Adjusted[a]**	
Any viral infection														
Positive	5	(1.7)	11	(4.7)	2.91	(1.00-8.49)	3.32	(0.93-11.8)	10	(3.7)	2.28	(0.77-6.77)	2.21	(0.71-6.84)
Negative	296	(98.3)	224	(95.3)	ref		ref		259	(96.3)	ref		ref	
Parvovirus B19[b]														
Positive	3	(1.0)	7	(3.0)	3.06	(0.78-12.0)	3.76	(0.77-18.3)	7	(2.6)	2.66	(0.68-10.4)	2.66	(0.64-11.1)
Negative	294	(97.7)	224	(97.0)	ref		ref		258	(97.4)	ref		ref	
HHV 6														
Positive	2	(0.7)	4	(1.7)	2.59	(0.47-14.2)	2.52	(0.33-19.5)	2	(0.7)	1.12	(0.16-8.00)	1.08	(0.14-8.08)
Negative	299	(99.3)	231	(98.3)	ref		ref		267	(99.3)	ref		ref	

[a] Adjusted for maternal age and number of previous births

[b] Not analysed in 8 cases (4 miscarriages and 4 preterm births) and 4 controls

PAPER III

Among women who previously had experienced pregnancy losses, the rate of preterm delivery was increased. In the multivariate analyses, a previous pregnancy loss was foremost associated with increased risk of extremely (≤ 27 weeks) and very (28-31 weeks) preterm deliveries (Table 3). Compared with women with no spontaneous abortions, the risk of extremely preterm delivery was more than doubled if the woman had one previous spontaneous abortion and quadrupled if she had had at least two spontaneous abortions. Similarly, if the woman had a history of one spontaneous abortion, there was a 50% increase in the risk of very preterm delivery, while two or more spontaneous abortions more than doubled the risk. Women with one or more previous missed abortions were at increased risk of preterm delivery, and the risk increase tended to be greater for extremely (OR=1.9) and very (OR=1.5) preterm delivery compared to moderately preterm delivery (OR=1.3). A history of ectopic pregnancies was also associated with increased risk of delivery before 32 weeks of gestation.

Table 3. Adjusted [a] odds ratios (OR) and 95% confidence intervals (CI) for preterm delivery in primiparous women associated with the woman's history of spontaneous abortions, missed abortions or ectopic pregnancies. Primiparous women with live singleton births in Sweden 1987-2000 (n=601 883).

	Extremely preterm delivery (≤ 27 weeks)	Very preterm delivery (28-31 weeks)	Moderately preterm delivery (32 -36 weeks)
Variable	**OR (95 % CI) [a]**	**OR (95 % CI) [a]**	**OR (95 % CI) [a]**
Number of spontaneous abortions			
0	1.0	1.0	1.0
1	2.2 (1.7-2.8)	1.5 (1.2-1.7)	1.1 (1.1-1.2)
≥ 2	4.0 (2.3-7.1)	2.7 (1.8-4.0)	1.6 (1.3-1.9)
Number of missed abortions			
0	1.0	1.0	1.0
≥ 1	1.9 (1.2-3.1)	1.5 (1.1-2.0)	1.3 (1.1-1.4)
Number of ectopic pregnancies			
0	1.0	1.0	1.0
≥ 1	1.5 (0.9-2.5)	1.6 (1.2-2.2)	1.1 (0.9-1.2)

[a] Adjusted for maternal age, smoking, relationship with father, mother's country of birth, and birth year.

Next, we stratified the analyses by cause of preterm delivery (Table 4). Compared with women with no spontaneous abortions, women with one previous spontaneous abortion had an almost doubled risk of delivery before 32 weeks due to preterm PROM, and a history of at least two spontaneous abortions was associated with a four-fold increase in risk. Prior spontaneous abortion also increased the risk of delivery before 32 weeks due to preterm labor, and the risk increased with number of spontaneous abortions. Compared with women with no missed abortion, a history of at least one missed abortion increased risks of preterm PROM and preterm labor. If the woman previously had had an ectopic pregnancy, the risk of preterm labor was increased. Previous pregnancy losses were generally not associated with other causes of delivery before 32 weeks.

When we studied risks of moderately preterm deliveries (32-36 weeks), a similar pattern was obtained, although the risks were not as prominent as for deliveries before 32 weeks (data not shown).

Table 4. Rates and adjusted[a] odds ratios (OR) and 95% confidence intervals (95% CI) of delivery before the 32nd week of gestation due to preterm PROM[b], preterm labor and other diagnoses in relation to the woman's history of spontaneous abortions, missed abortions or ectopic pregnancies.

	Preterm PROM (n=987)		Preterm labor (n=2 932)		Other diagnoses (n=1 115)	
Variable	**(%)**	**OR (95% CI)[a]**	**(%)**	**OR (95% CI)[a]**	**(%)**	**OR (95% CI)[a]**
Number of spontaneous abortions						
0	(0.2)	1.0	(0.5)	1.0	(0.2)	1.0
1	(0.3)	1.9 (1.5-2.6)	(0.7)	1.7 (1.4-2.0)	(0.3)	1.4 (1.0-1.9)
≥ 2	(0.7)	4.1 (2.2-7.8)	(1.6)	3.2 (2.1-4.9)	(0.2)	1.3 (0.4-4.1)
Number of missed abortions						
0	(0.2)	1.0	(0.5)	1.0	(0.2)	1.0
≥ 1	(0.4)	2.2 (1.4-3.7)	(0.9)	1.6 (1.1-2.2)	(0.2)	0.9 (0.4-2.1)
Number of ectopic pregnancies						
0	(0.2)	1.0	(0.5)	1.0	(0.2)	1.0
≥ 1	(0.4)	1.4 (0.8-2.6)	(0.9)	1.5 (1.1-2.1)	(0.3)	1.9 (1.0-3.5)

[a] Adjusted for maternal age, smoking, relationship with father, mother's country of birth, and birth year.
[b] Preterm premature ruptures of the membranes

PAPER IV

A previous preterm delivery was not associated with an increased risk of autism in the offspring, neither where previous pregnancy losses, difficulties to conceive before present pregnancy, maternal infections during pregnancy, season of delivery or diabetes (Table 5). Preeclampsia, but not gestational hypertension, was associated with an increased risk of autism.

Table 5. Distribution of maternal and pregnancy characteristics as well as neonatal complications, and associated risks, among cases with autism and the controls.

Maternal and pregnancy characteristics	Cases (N=1216)		Controls (N=6080)		Odds ratio (95% CI)			
	N	(%)	N	(%)	Crude[a]		Adjusted[b]	
Parity								
0	467	(40.6)	2479	(42.4)	1.00		1.00	
≥ 1, with no previous preterm delivery	621	(53.9)	3107	(53.2)	1.07	(0.93-1.22)	1.06	(0.91-1.23)
≥ 1, with at least one previous preterm delivery	63	(5.5)	259	(4.4)	1.30	(0.97-1.74)	1.29	(0.93-1.78)
Missing	65		235					
Previous miscarriage								
No	1030	(84.7)	5230	(86.0)	1.00		1.00	
Yes	186	(15.3)	850	(14.0)	1.11	(0.94-1.32)	1.08	(0.89-1.32)
Childless years								
No	1158	(95.4)	5757	(95.1)	1.00		1.00	
≥1	54	(4.5)	299	(4.9)	0.90	(0.67-1.21)	0.78	(0.55-1.11)
Missing	4		24					
Any maternal infection during pregnancy								
No	1188	(97.7)	5957	(98.0)	1.00		1.00	
Yes	28	(2.3)	123	(2.0)	1.15	(0.75-1.75)	1.04	(0.63-1.71)
Season of delivery								
Jan-April	392	(32.2)	2182	(35.9)	0.85	(0.74-0.97)	0.84	(0.72-0.98)
May-December	824	(67.8)	3898	(64.1)	1.00		1.00	
Diabetes[c]								
No	1198	(98.5)	6000	(98.7)	1.00		1.00	
Yes	18	(1.5)	80	(1.3)	1.13	(0.67-1.89)	0.90	(0.49-1.67)
Hypertensive disease								
No	1156	(95.1)	5850	(96.2)	1.00		1.00	
Gestational hypertension	21	(1.7)	90	(1.5)	1.18	(0.73-1.92)	1.04	(0.59-1.81)
Preeclampsia	39	(3.2)	140	(2.3)	1.41	(0.98-2.02)	1.64	(1.08-2.49)

[a] Adjusted for age, sex, birth year and birth hospital through matching. Observations with missing values on the variable are not included in the analyses.

[b] Matched analysis, all variables are also adjusted for each other as well as for maternal age, smoking, maternal country of birth, if the mother lived with the father or not, and maternal schizophrenia. 833 observations were not included in the analyses due to missing information on any of the covariates adjusted for.

[c] Includes both pregestational and gestational diabetes

Several birth characteristics and neonatal complications were associated with increased risk of autism (Table 6). Being small-for-gestational-age and congenital malformations doubled the risk of autism, while there was no increase in risk for twins. Intracranial bleeding, seizures or edema in the neonatal period were associated with a more than tripled risk of autism. Neonatal hypoglycemia was also associated with an increased risk of autism. As expected, almost all pregnancy, birth and neonatal complications were more common among preterm than term born infants.

Table 6. Distribution of infant characteristics and neonatal complications, and associated risks, among cases with autism and the controls.

Infant characteristics and neonatal complications	Cases (N=1216)		Controls (N=6080)		Odds ratio (95% CI)			
	N	(%)	N	(%)	Crude[a]		Adjusted[b]	
Birth weight for gestational age								
SGA	66	(5.4)	147	(2.4)	2.36	(1.75-3.19)	1.86	(1.32-2.63)
AGA	1095	(90.0)	5709	(93.9)	1.00		1.00	
LGA	55	(4.5)	224	(3.7)	1.28	(0.94-1.73)	1.18	(0.84-1.66)
Congenital malformations								
Yes	97	(8.0)	232	(3.8)	2.17	(1.70-2.77)	2.06	(1.56-2.71)
No	1119	(92.0)	5848	(96.2)	1.00		1.00	
Twin								
Yes	38	(3.1)	153	(2.5)	1.25	(0.87-1.79)	1.27	(0.86-1.91)
No	1178	(96.9)	5927	(97.5)	1.00		1.00	
Apgar score at 5 minutes								
0-6	32	(2.7)	73	(1.2)	2.22	(1.46-3.38)	1.39	(0.84-2.29)
7-10	1171	(97.3)	5929	(98.8)	1.00		1.00	
Missing	13		78					
Intracranial bleeding, cerebral edema or seizures								
Yes	25	(2.1)	28	(0.5)	4.46	(2.60-7.66)	3.06	(1.56-5.99)
No	1191	(97.9)	6052	(99.5)	1.00		1.00	
Delivery associated head and neck injuries								
Yes	27	(2.2)	124	(2.0)	1.09	(0.72-1.66)	1.03	(0.65-1.63)
No	1189	(97.8)	5956	(98.0)	1.00		1.00	
Any neonatal infections								
Yes	52	(4.3)	158	(2.6)	1.67	(1.22-2.31)	1.33	(0.92-1.95)
No	1164	(95.7)	5922	(97.4)	1.00		1.00	
Hypoglycemia								
Yes	41	(3.4)	73	(1.2)	2.93	(1.98-4.35)	2.20	(1.39-3.49)
No	1175	(96.6)	6007	(98.8)	1.00		1.00	
Respiratory distress								
Yes	58	(4.8)	206	(3.4)	1.46	(1.08-1.96)	1.00	(0.70-1.43)
No	1157	(95.2)	5874	(96.6)	1.00		1.00	
Neonatal jaundice								
Yes	74	(6.1)	285	(4.7)	1.32	(1.01-1.72)	1.18	(0.86-1.63)
No	1142	(93.9)	5795	(95.3)	1.00		1.00	

[a] Adjusted for age, sex, birth year and birth hospital through matching.

[b] Matched analysis, all variables are also adjusted for each other as well as for maternal age, smoking, maternal country of birth, if the mother liver with the father or not, and maternal schizophrenia. 91 observations were not included in the analyses due to missing value on Apgar score at five minutes.

Compared with children born at term (37 – 41 weeks), the risk of autism was increased by 50 percent in children born moderately preterm (32-36 weeks) and doubled for those born very preterm (≤31 weeks), when only adjusting for the matching variables (Table 7, crude model). When maternal and pregnancy characteristics were included as covariates, these risks were slightly attenuated (Table 7, adjusted model 1), mostly due to the effect of preeclampsia. Adjusting for infant characteristics resulted in a further attenuated and non-significant association (Table 7, adjusted model 2), and after adjusting for neonatal morbidity we no longer found associations between preterm birth and risk of autism (Table 7, adjusted model 3).

Next, we studied if the risk of autism related to birth characteristics and neonatal complications differed between preterm and term born children. Both respiratory distress and neonatal jaundice modified the effect of gestational age on risk of autism (P-values for interactions were 0.00 and 0.02, respectively). In term born children, respiratory distress and neonatal jaundice were associated with increased risk of autism, while there was no increase in risk among preterm born children. Neonatal hypoglycemia was associated with a three-fold increased risk of autism in children born at term, while no increase in risk was observed among those born preterm (P-value for interaction was 0.08).

Table 7. Gestational age at delivery and adjusted odds ratios of autism.

	Cases (N=1216)		Controls (N=6080)		Odds ratio (95% CI)							
	N	(%)	N	(%)	Crude model[a]		Adjusted model 1[b]		Adjusted model 2[c]		Adjusted model 3[d]	
Gestational weeks at delivery												
≤ 31	23	(1.9)	59	(1.0)	2.03	(1.13-3.64)	1.88	(1.00-3.53)	1.48	(0.77-2.84)	0.98	(0.45-2.16)
32-36	96	(7.9)	324	(5.3)	1.52	(1.16-1.99)	1.44	(1.07-1.94)	1.33	(0.98-1.81)	1.25	(0.90-1.75)
37-41	1000	(82.4)	5207	(85.9)	1.00		1.00		1.00		1.00	
≥ 42	95	(7.8)	474	(7.8)	1.13	(0.88-1.45)	1.18	(0.92-1.53)	1.18	(0.91-1.53)	1.12	(0.86-1.45)
Missing	2		16									

[a] Adjusted for sex, birth year, birth hospital and date of diagnosis through matching. 18 observations were not included in the analyses due to missing values on gestational age.
[b] Matched analysis, also adjusted for maternal age, smoking, maternal country of birth, if the mother lived with the father or not, maternal schizophrenia and for maternal and pregnancy characteristics (previous miscarriage, previous preterm delivery, difficulties in conceiving, maternal infections, season of birth, preeclampsia and maternal diabetes). 1122 observations were not included in the analyses due to missing values on any of the variables.
[c] Matched analysis, also adjusted for all variables included in Model 2 as well as for the birth characteristics (SGA, congenital malformations and twin ship). 1122 observations were not included in the analyses due to missing values on any of the variables.
[d] Matched analysis, also adjusted for all variables included in Model 3 and for the neonatal complications (low Apgar score, intracranial bleeding, seizures or edema, delivery-associated head or neck injuries, neonatal infections, hypoglycemia, respiratory distress or neonatal jaundice). 1188 observations were not included in the analyses due to missing values on any of the variables.

DISCUSSION

METHODOLOGICAL CONSIDERATIONS

Study design

Two commonly used study designs within epidemiological research are case-control and cohort studies. In cohort studies, the distribution of disease among exposed and non-exposed is studied, while in case-control studies, the distribution of exposure among persons with and without disease is assessed.

Cohort studies (Studies I and III)

In classical cohort studies, so called prospective cohorts, a population, including both exposed and non-exposed individuals, is followed over time, and information regarding exposures and outcomes are collected prospectively. There are also retrospective cohort studies; these are often based on register data, i.e., data which has already been collected. In other words, in a prospective cohort, the outcome has not yet occurred when the study is launched, while in a retrospective cohort study the outcome has occurred when the study is initiated, although data may still be prospectively collected. Further, a cohort can be both open and closed. In an open cohort, new members are allowed to enter and leave the cohort, while this is not the case in a closed cohort.

One advantage of cohort studies is that the study design makes it possible to study multiple effects of a single exposure. It is also possible to study temporal relationship between exposure and outcome as well as allowing direct measurements of incidence in both the exposed and the unexposed groups. The cohort study can also be the optimal design to study rare exposures, where exposed individuals and a control group of unexposed individuals are followed over time. The main disadvantages are that cohort studies often are very expensive and time consuming. Further, if the outcome is rare, a very large cohort is needed. In addition, loss to follow-up can affect validity. A number of these disadvantages only apply to prospective cohort studies. Retrospective cohort studies have the advantages of being both quick and cheap, but are limited to the exposures and outcomes on which information has been collected. Also, since retrospective cohort studies are based on already collected information it is difficult to improve the quality of data.

In this thesis, studies I and III are retrospective cohort studies, based on registers with prospectively collected data. In both study I and III, information on exposure and outcome was collected from the population-based Medical Birth Register, which includes 99% of all births in Sweden. In study I, exposure was also obtained from The Pap Smear Screening Register, which contains all Pap smears taken during pregnancy in the counties of Uppsala and Gävleborg during the study period. In study III, information about the main exposures was obtained from the Hospital Discharge Register, covering all hospital admissions during the study period. These study designs give us the advantage of a very large study sample, which provides possibilities to study rare outcomes with high precision. Also, the prospective data collection results in good internal validity.

Case-control studies (Papers II and IV)
In case-control studies a number of cases (with disease) and a number of controls (without disease) are selected. The difference with regards to a certain exposure is then assessed between the two groups.

Case-control studies also have a number of strengths and limitations. As opposed to cohort studies, the case-control design is good when studying rare diseases or diseases with long induction time. It is also possible to study several exposures, and the studies are generally cost- and time-efficient. Limitations of case-control studies are that they are not suitable for very uncommon or very common exposures since we want to maximize the variation in exposure between cases and controls. By definition, it is not possible to study several outcomes. A crucial aspect of case-control studies concerns the selection of cases and controls. Controls should be selected in such a way that they were eligible as cases if they had developed disease. It is further important that the distribution of exposure among the controls represents the source population, to ensure a high external validity. A case-control study can be matched, in which cases and controls are matched to each other by important factors (confounders). By matching the efficiency in the analysis is improved, but it is no longer possible to estimate the effect of the matching factor. A case-control study may also be nested, in which the cases and controls are selected from a well-defined cohort of exposed and unexposed individuals. This is a way of mimicking a cohort study without using the entire cohort. Since fewer individuals are needed, the efficiency is usually improved. Another advantage of a nested case-control study is that the absolute risk may be reliably estimated.

Studies II and IV are case-control studies, which are both nested in population-based cohorts. In study II, blood samples were retrieved from a number of women who later had a miscarriage or a preterm delivery (cases) and women who had a term delivery (controls). The blood samples were then analyzed with regards to viremia. Since we wanted to study several exposures and also due to the fact that the laboratory analysis of blood is costly, a case-control study, nested within a population-based cohort, was preferred. In study IV, we wanted to study only a single outcome, autism, which is fairly rare. We also wanted to assess the effect of several exposures on that outcome. Since we used prospectively collected data from existing registers, we could have performed a retrospective cohort study. However, a nested case-control study has the same methodological strengths.

Internal validity

Within epidemiological studies there are two common types of error; random errors and systematic errors. Random errors can usually be avoided by increasing sample size, while systematic errors are not affected by study size. Systematic errors are often referred to as biases and can be introduced at any stage of the study. Two important biases are *selection bias* and *information bias*. Systemic error may also be introduced due to some third factor affecting both exposure and outcome, so called *confounding*.

Selection bias

Selection bias is introduced when the association between exposure and outcome differs for those who are selected to participate in the study and for those who are not selected. Since we usually do not know the association between exposure and outcome in the group who do not participate, selection bias can often not be observed. Instead, selection bias is rather something we need to evaluate and consider when conducting studies. For example, if we want to study the effect of maternal smoking during pregnancy on risk of preterm birth and we chose all cases from a university clinic and all controls from a small-town clinic we may introduce selection bias. We may argue that women at the university clinic and the small-town clinic differ with regard to both smoking prevalence and rates of preterm delivery.

Selection bias is mainly a problem in case-control studies, but may propose a problem in retrospective cohort studies as well. In retrospective cohort studies it is introduced when selection of exposed and non-exposed subjects are related to the development of the outcome. Generally, prospective collection of data minimizes the risk of selection bias.

In study II, exposure was assessed from blood samples obtained in the Rubella screening program offered to all pregnant women in Sweden when registering to antenatal care in early pregnancy. The preterm cases and controls were selected from the Medical Birth Register, covering 99% of all newborns and we therefore find it highly unlikely that selection bias would be a problem. We do not know what proportion of women in Stockholm County with a second trimester miscarriage were admitted to hospital for inpatient care during the time of the study (1990-2002). This may have introduced a selection bias of cases, and spontaneous abortions with longer gestational ages may be over-represented.

In study IV, the selection of controls is not likely to cause a problem, since all controls were chosen from the Medial Birth Register. However, the selection of cases may be a concern. The study only included children with autism that had been admitted to hospital, which is likely to affect the representativeness of the autism cases and probably severe cases of autism are over-represented. If associations between exposures and autism differ by disease severity, the results may not be applicable to milder forms of autism. Since cases could also have be admitted under a secondary diagnosis of autism we most likely also have an over-representation of children with other diseases, and we might suspect that the associations found between neonatal complications and autism are over estimations of the true association.

Information bias

The term information bias, or misclassification bias, refers to when information regarding the study subjects in a study has been misclassified. This misclassification may be differential or non-differential. A differential exposure misclassification is one where the misclassification is different for those with and without disease. If the misclassification of exposure is independent of disease it is referred to as non-differential. While a differential misclassification may lead to both over- and underestimations of the association between exposure and outcome, a non-differential misclassification will (if anything) only dilute the risks, i.e. bias the results towards the null.

One type of misclassification is recall bias, which refers to the fact that cases and controls may recall information differently. In this thesis, no information has been retrospectively collected from cases or controls, which should preclude the risk of recall bias.

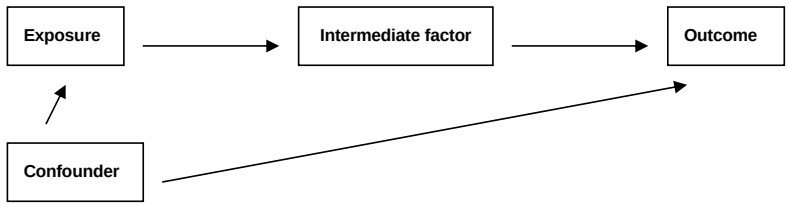
In study I, the information on infections was assessed by personnel investigating Pap smears before the outcomes occurred. Thus, although some misclassification probably occurred, it is not likely that this misclassification differed with regards to pregnancy outcomes.

In study II exposure to viremia was measured at a single point in early pregnancy. Since viremia may be short-lived there is a risk of misclassification, but this is most likely non-differential with regard to the outcome and would therefore, if anything, dilute our results towards the null.

In study III, the information on miscarriages was retrieved from the Hospital Discharge Register. Since a number of miscarriages are subclinical and not all clinically recognized miscarriages are treated with inpatient care, this proposes a limitation. The main indication for in-patient care of the miscarriage is gestational length, with later miscarriages being treated more often as in-patients. But the misclassification is most likely non-differential since there is no reason to believe that there would be a difference with regard to in-patient care of miscarriages among women who later deliver preterm or term.

Confounding

Confounding is a central concept within epidemiology and refers to when the association between exposure and outcome is affected by a third variable. This third variable, the confounder, explains all or part of the association between exposure and outcome. For a variable to be considered a confounder it has to be related to both exposure and outcome and further should not be in the causal pathway between exposure and outcome. If a factors lies in the causal pathway between exposure and outcome it is regarded as a potential intermediate factor.



The issue of confounding can be dealt with in a number of ways. In cohort studies, it is important that, as far as possible, the unexposed resembles the exposed in all aspects except for exposure. Restricting the study subjects to a homogeneous group may limit the influence of possible confounders. In case-control studies, cases and controls can be matched to each other with regard to potential confounders. Experimental studies are regarded as the ideal study situation, in which randomization into exposed and non-exposed groups will lead to an even distribution of confounders. Finally, it is possible to adjust for the effect of potential confounders by conducting multivariate analyses.

In study I, which covered the years 1973 through 2000, we conducted multivariate analyses, controlling for maternal age, parity, previous preterm delivery, and county of test. To control for differences in measurements of gestational length during the study period, we adjusted for calendar year when the test was performed. Another possible confounder in study I is smoking. Unfortunately we did not have information on maternal smoking for the entire study period, and could therefore not include this variable in the analyses.

In study II data on miscarriages was retrieved from the Hospital Discharge Register and information on gestational length and parity was retrieved from medical records. For miscarriage cases, we did not have information on possible confounders, such as smoking or BMI. Among cases with preterm delivery, we also had a large number of missing information on the variables BMI and smoking. This combined with the generally low power due to samples older than ten years having been cleared from the lab, made us unable to adjust for smoking and BMI also in the group with preterm cases.

In study III, the study period was 1987 to 2000, and we had better information on a number of potential confounders, including smoking. In the multivariate analyses we were therefore able to adjust for maternal age, smoking, if the mother cohabited with the father or not, maternal country of birth and birth year.

In study IV, we wanted to investigate the risk of autism with respect to infectious-related exposures and preterm birth. Specifically, we wanted to investigate whether the previously found relationship between preterm birth and autism could be explained by potential confounders or intermediate factors related to preterm birth. Cases and controls were matched with regard to sex, birth year and birth hospital. We had information on a number of potential maternal confounders (maternal age, smoking, cohabitation with the father or not, and maternal schizophrenia). We also had information on pregnancy related factors (parity and previous preterm delivery,

previous miscarriage, involuntary childlessness, maternal infections, season of delivery, maternal diabetes, and hypertensive disease during pregnancy), which could be possible confounders. Further, we had information on infant characteristics (SGA, congenital malformations, twinning) and neonatal complications (Apgar score, intracranial bleedings, neonatal infections, hypoglycemia, respiratory distress and neonatal jaundice), which may act as intermediate factors between preterm delivery and autism. In an attempt to explain the relationship between preterm birth and autism we adjusted for all potential confounders and intermediate factors in a stepwise manner. A possible case of confounding could arise if the probability of being diagnosed with autism as an inpatient was linked to the exposures measured, for example if a preterm born child showing symptoms of autism is more or less likely to be evaluated as an in-patient compared to a term born child with the same symptoms and being alike in all other aspects.

Effect modification and interaction

Effect modification refers to the situation in which a measure of effect changes over the value of some other variable. That is, the effect of a certain exposure on an outcome is not the same depending on the value of a third variable.

In the fourth study we found that the association between some neonatal exposures and risk of developing autism was significantly different between preterm and term born children. This is referred to as effect modification or interaction. If effect modification is detected, this needs to be taken into consideration, either by stratifying the analyses on the depending variable or by conducting an interaction analysis where the variation by this third variable is considered. In the fourth study, we conducted interaction analyses and received stratum specific odds ratios.

Random errors

Random errors can occur in all studies and can lead to results that are due to chance. Within epidemiological research, the use of statistical methods in assessing risk estimates and calculating confidence intervals aims at reducing random findings. The confidence limit is usually set to 95 percent. This means that when the confidence limit for the risk estimate does not include 1.00 there is a 95 percent probability that the result is not due to chance.

One way to reduce problems with random errors when designing a study is to increase the study size. In study I, III and IV the study sizes were all reasonably large, giving us reliable estimates. In study II, we lost a number of cases and controls after the study was initiated. Besides, the final analysis was based on a smaller sample size than was initially planned; the prevalence of viral infections was also unexpectedly low. Since this substantially reduced the statistical power of the study, the estimates may have been influenced by effects of random errors.

External validity

External validity or generalizability refers to whether the results found within a study may be applicable to a general population. There is no point in being concerned about external validity if the internal validity is not good. That is, if the results within a study are not trustworthy, it is meaningless to consider if they apply to other non-studied subjects. Whether a study has high external validity depends on both biological mechanisms and representativeness of the studied subjects. Thus it must be probable that the association found is biologically plausible also in other non-studied subjects. In addition, the sampling from the source population should have been done is such a way that the studied subjects contain a fair sample of the source population. Whether the results can be extrapolated to other populations is a discussion which should be based on the degree of similarity between the studied and the non-studied populations.

In study I, one might question which women had a Pap smear taken during pregnancy, and if these women differ with regard to the general population in some sense which would also be linked with the outcome. Pap smears are primarily taken as a part of routine screening for cervical cancer, and is offered to all Swedish women between the ages of 23 and 60 every third to fifth year. Our study cohort (women who had a Pap smear taken during pregnancy) comprises approximately 50% of the pregnant population during the study period, and did not differ significantly from all pregnant women in the county with regard to maternal characteristics or pregnancy outcomes. We therefore find it unlikely that the results found would not be applicable to a general population.

Since the internal validity of study II is questionable due to its small sample size, the external validity may propose a problem. In study III, the study sample is large and there is no reason to believe that the results are not generalizable to a pregnant population with similar characteristics. The internal validity of study IV is good. However, the study only included children with autism that had been admitted to hospital, which makes it likely that severe cases of autism are over-represented. If associations between exposures and autism differ by disease severity, the results may not be applicable to milder forms of autism.

FINDINGS AND IMPLICATIONS

Infections and risk of miscarriage or preterm delivery (Study I, II and III)

The causes of preterm birth are not uniform throughout gestation. For example, bacterial infection is a rare cause of moderately preterm delivery (32-36 weeks), but probably a main cause of deliveries before 30 weeks[71, 146]. There has also been an association with bacterial infections and late miscarriages[35, 87, 147]. Much less is known about the association between viral infections and miscarriage or preterm delivery.

For both miscarriages and preterm deliveries a repetitive pattern has been observed, that is women with a miscarriage has an increased risk of miscarriage in the subsequent pregnancy and the same is seen with very preterm deliveries[57, 58]. Second trimester miscarriage also increases subsequent risk of very preterm delivery[148]. It has therefore been postulated that late miscarriages and early preterm deliveries share etiological causes: both may be caused by genital infections, which are believed to ascend through the vagina and cervix and thereby cause spontaneous preterm labor and/or preterm premature rupture of the membranes. With this hypothesis, women who are more susceptible to genital infections and/or carry chronic genital infections would be at greater risk of both miscarriage and preterm delivery.

Bacterial infections, miscarriage and preterm delivery (Study I and III)

Findings: In study I, we found no association between infectious signs at a non-specific time in pregnancy and risk of preterm birth. Presence of Coccobacilli in Pap smear taken within four weeks before delivery was associated with a four-fold increase in risk of preterm delivery before the 32nd week of gestation and also increased the risk of delivery of a SGA infant. The presence of Coccobacilli on Pap smear has in other studies been determined to be a good diagnostic criterion of Bacterial vaginosis[91, 92] and our results are therefore in accordance with studies on Bacterial vaginosis and risk of preterm delivery[35].

Only a few studies, with conflicting results, have previously been made on the association between infections signs in Pap smears and increased risk of preterm delivery[78, 149-151]. The studies made by Lanouette et al.[149] and Blake et al.[150] found no association between inflammations in Pap smears and preterm delivery, whereas a later study by Mass et al.[151] found that presence of an altered vaginal flora on Pap smears increased the risk of preterm delivery 2.5 times. Jacobsson et al. found no significant association between Bacterial vaginosis in early pregnancy and increased risk of spontaneous preterm delivery although Bacterial vaginosis was more frequent among women delivering preterm[78]. These conflicting results may be explained by the fact that the studies conducted were relatively small and limited to Pap smears taken in early pregnancy. Furthermore, either mean gestational age has been compared between women with or without signs of infection, or preterm delivery has been classified as being born before the 37th gestational week. As genital infections have primarily been associated with very preterm delivery, these definitions may have influenced the results of the studies[72, 146].

In the third study we found that a previous miscarriage increased the risk of preterm delivery in the subsequent pregnancy and that this risk increase was mainly seen in extremely and very preterm deliveries. The risk related to a previous miscarriage was also limited to very preterm deliveries with a spontaneous onset. Since urogenital infections have been associated with both second trimester miscarriages and very preterm deliveries, this association may be due to women who are more susceptible to infections or carrying chronic infections are at increased risk of both miscarriage and preterm delivery.

Interpretation:
The results in study I may be interpreted in different ways: either is the risk of preterm delivery associated with Bacterial vaginosis limited to the second trimester or we were, due to methodological issues, unable to detect the association at a non-specific time in pregnancy. A study by Mass et al[151] showed that only women who tested negative at 24 weeks but positive at 28 weeks were at increased risk of a spontaneous preterm delivery, indicating that a recent infection may imply a higher risk than a chronic infection. A woman with symptoms of Bacterial vaginosis in early pregnancy may also be treated and thereby lower her risk of preterm delivery. Since the general policy in Sweden is to treat Bacterial vaginosis if detected during pregnancy this could explain why we did not see an association between infection at a non-specific time in pregnancy and risk of preterm delivery. An alternative explanation is that the low sensitivity to detect Bacterial vaginosis and other infectious signs on Pap smear makes it difficult to find an association at a non-specific time in pregnancy. Further, it might also be that the found association between Bacterial vaginosis and increased risk of very preterm delivery is due to some unmeasured confounder. One such possible confounder is smoking, which could be associated with increased risk of both infections and preterm delivery, in particularly very preterm delivery[40, 42]. Another factor which could also help us to interpret our results would be if we had information on how the delivery was initiated, since we believe that presence of Bacterial vaginosis would mainly be associated with increased risks of spontaneous onset of delivery.

In study III, maternal smoking during pregnancy did not confound the association between previous miscarriages and subsequent risk of preterm delivery. Since we are studying different exposures in study I and III this does not have to apply to study I. In study III we also had information on onset of delivery and found that the increase in risk for very preterm deliveries was limited to deliveries with a spontaneous onset. This supports the hypothesis of an infectious aetiology in preterm deliveries associated with previous pregnancy losses.

A weakness in study III is that information on previous miscarriages was collected from the Inpatient Register and we therefore lack information on gestational lengths of miscarriages. However, we have information of gestational age from spontaneous and missed abortions among women admitted to in-patient care from 1990-2000 in two Swedish hospitals. The data showed that 77% of the pregnancy losses occurred in the first trimester and 23 % in the second trimester. Of all pregnancy losses, 56% occurred at 11-14 gestational weeks. If this distribution of gestational lengths among miscarriages admitted for hospital care also applies to our study, our results can be interpreted in two different ways: the association between spontaneous abortion and

subsequent risk of preterm delivery is not limited to second trimester abortions and/or the true association is underestimated due to selection bias.

Previous studies have primarily found an association between previous pregnancy loss in the second trimester and preterm delivery[35, 152, 153]. Some, but not all first trimester pregnancy losses have abnormal karyotype. A study of fetal losses with known karyotype before the 13th gestational week showed that 60% had abnormal fetal karyotype, while 40% had normal fetal karyotype[154]. Therefore, although infections are generally regarded as the main cause of second trimester losses, infections may possibly also cause late first trimester losses of normal fetal karyotype.

Although different exposures were used in study I and III, the results still point in the same direction, supporting the hypothesis that infections or infectious related morbidity during pregnancy have its greatest impact during the second trimester. However, whether treatment of Bacterial vaginosis detected during the second trimester actually lowers the woman's risk of very preterm delivery remains to be determined[93, 94].

Viral infections and risk of miscarriage or preterm delivery (Study II)

Findings: Although no significant results were obtained at the 5% level in study II, all the point estimates were elevated. The study was hampered by low statistical power. Still the results may indicate an increased risk of second trimester miscarriage and very preterm birth among women with viral infection in early pregnancy.

Interpretation: We found no association between viral infection and risk of spontaneous preterm delivery. This could be due to viral infections inducing preterm birth by another mechanism than preterm labour or premature rupture of the membranes. An alternative explanation is that we had too low statistical power in our study to detect a possible association.

The mechanism by which viral infection may cause miscarriage or preterm delivery is not known, but it may be related to general inflammation of the mother or foetus. Women with preterm delivery have been found to have an increase in inflammatory markers such as IgM and CRP, and inflammatory changes of the placenta have also been found in women with preterm delivery[61, 155-157].

Further, mechanisms related to placental dysfunction have been suggested to be related to preterm birth. In pregnancies with adverse outcome and diagnosed Parvovirus B19 infection, significantly increased apoptosis has been found in placental villous throphoblast cells[158]. Preeclampsia, a condition closely related to preterm birth, has also been found to be linked to Cytomegalovirus, with increased levels of antibodies against CMV in women with early onset preeclampsia[104]. In our study the prevalence of CMV was unexpectedly low, we only detected one case in a women who delivered preterm. The only other herpes virus detected in our study was HHV6, and our findings indicate a possible association between HHV6 infection and increased risk of second trimester miscarriage, but not very preterm birth. To our knowledge only two previous studies[97, 146], have been conducted on HHV6 and foetal loss, with contradictory results.

In our study, the point estimates of risk were, if anything, larger for second trimester miscarriages compared to very preterm deliveries. In study III, we found that women with previous miscarriages were at increased risk of preterm delivery in the subsequent pregnancy[159] and that the increased risk was inversely related to gestational length. This indicates a possible shared aetiology between miscarriage and very preterm delivery. Other studies have also found that miscarriage and very preterm delivery share risk factors, such as smoking, socioeconomic status and maternal age[41, 57, 58]. It could also be that the fetus is more vulnerable to certain infections during the second trimester[160]. Parvovirus B19 is believed to infect the liver of the fetus, thereby causing severe anaemia, congestive heart failure and development of hydrops fetalis[161]. Since the liver is the main source of haematopoietic activity during the second trimester, this is the period during which the fetus is most vulnerable for liver infections[160].

In conclusion, our results suggest that viral infections with Parvovirus B19 and Human Herpes virus in early pregnancy may be associated with increased risk of second trimester miscarriage and very preterm delivery. If confirmed in larger epidemiological studies, our findings may contribute to further knowledge on the biological mechanisms related to second trimester miscarriages and very preterm birth. Such knowledge may also in the long run be important for developing strategies to prevent early pregnancy loss, since viral infections are principally preventable diseases.

Infections, preterm birth and associations with Autism (Study IV)

Findings: In study IV we found no association between infections or infectious signs in pregnancy and later risk of autism. Instead we found that preterm born children were at increased risk of developing autism and that this association was explained by maternal morbidity, birth characteristics and neonatal complications related to preterm birth. This indicates that the association between preterm birth and risk of autism may not be due to short gestational age per se, but rather be mediated by prenatal and neonatal complications influencing brain development, such as intracranial bleedings or deviations from a normal fetal growth curve.

Interpretation: When reviewing the literature on autism there is not much evidence of an association between viral infections and later risk of autism. It should however be emphasized that the field is complicated to investigate. Viral infections may be asymptomatic and the sensitivity to detect infections may therefore be low. Autism is also a rare disease with a wide spectrum of symptoms.

In our study we received information on infections during pregnancy from the Medical Birth Register and we strongly suspect that we have a low sensitivity. Further, we used a number of proxies for infection such as a previous preterm delivery or miscarriage, difficulties to conceive and season of birth. Since these are only indirect measurements of infection we are likely to have a number of misclassifications. We therefore need to be cautious when interpreting the negative findings. Still, previous positive findings of associations between prenatal infections and autism have primarily been found with Rubella[123, 129], which is practically eradicated in Sweden. Vaccination against Rubella has been offered to all children in Sweden since 1974, and all pregnant women are screened for Rubella in early pregnancy since 1975.

A substantial amount of research has been conducted in the field of perinatal epidemiology and autism. It has been fairly established that the risk of autism increases with increasing maternal age and decreasing birth weight[114-116, 118, 119, 121, 162]. Further, congenital malformations and short gestational length are also associated with an increased risk of autism in the child[114, 118, 119, 162]. In conclusion, the research points at children born under certain conditions being at increased risk of autism. This is of particular importance from a public health perspective since it indicates which children could benefit from being monitored. Early detection and treatment of autism has shown to have a positive impact on symptom development.

During the last decades there has been an immense development of neonatal care which has led to a substantial decrease in mortality among preterm born children; and today, a majority of children born at 28th gestational weeks survive[33]. Since the rate of morbidity has remained almost the same, the decreased mortality has led to an increase in the absolute number of children with neonatal complications surviving into adulthood[33]. This has in turn led to an increasing interest in the long-term effects of preterm birth. Several studies have reported an increase in adverse long-term neurodevelopment among very preterm born children[163, 164], and the prevalence is also higher for psychiatric disorders like schizophrenia and anorexia nervosa among preterm born children[165, 166]. Further, very preterm born children are reported to have deficits in language and cognition. The medical and social consequences related to preterm birth have been found to be inversely related to gestational length[167, 168]. A study conducted in Belgium found that as many as 70% of children born before the 26th week displayed a mental or psychomotor impairment at 3 years of age[163], and a Swedish study on 10- to 12-year-old children born between the 23rd and 25th week found an increase in problems related to mental health and social competence[164].

The risk of neonatal complications and morbidities is far higher in preterm compared with term born infants[169]. It is not known whether the association found between preterm birth and autism is due to a general immaturity of the preterm born child or due to complications associated with very preterm birth.

In our study, very preterm birth was associated with a doubled risk of autism and being moderately preterm born increased the risk of autism by 50%. In an aim to elucidate the factors behind this association we adjusted for maternal, pregnancy and birth characteristics as well as for neonatal complications in a step wise manner. After adjusting for all factors, no association was found between preterm birth and increased risk of autism.

The main factor mediating the association between preterm birth and autism was presence of intracranial bleeding, cerebral edema or seizures in the neonatal period. Preterm born children followed with neuroimaging more often display a deviation from the normal brain structure, which has also been correlated with long-term neurodevelopmental problems. Intraventricular hemorrhage, a condition almost exclusively occurring in preterm born infants, increases the risk of long-term neurological deviations[170, 171], and two thirds of survivors of periventricular hemorrhage display significant cognitive or motor abnormalities. A recent study conducted on 86

preterm born children, showed that isolated cerebellar hemorrhagic insults in the neonatal period were associated with a high prevalence of long-term pervasive neurodevelopmental disabilities[141]. Further, changes in grey and white matter distribution in adolescents born very preterm have been found to be associated with cognitive impairment[172].

A low Apgar score at five minutes also influenced the association between preterm birth and autism. A low Apgar score is an indication of birth asphyxia and has previously been associated with increased risk of autism[114, 115, 118]. A low Apgar score is more common in preterm births and is a valuable risk indicator of hypoxic-ischemic encephalopathy[173].

We also found that preeclampsia in the mother during pregnancy, being born small-for-gestational age or having congenital malformations partly mediated the association between preterm birth and autism. Preeclampsia is characterized by placental dysfunction and often leads to an elective preterm delivery[174]. Previous studies conducted on the association between preeclampsia and autism have been inconclusive[114, 115, 118], but preeclampsia is most likely causally related to increased risk of intrauterine growth restriction[175]. The presence of a congenital malformation or poor fetal growth indicates a deviation from the normal developmental pattern during fetal life, circumstances which might affect fetal brain development. A deviation from the normal fetal growth curve infers that the fetus is exposed to suboptimal intrauterine conditions at some point and infants born small-for-gestational-age have generally higher risks of neurodevelopmental problems compared to children with normal birth weights[176, 177], and are also at increased risk for developing autism[118].

Interestingly, hypoglycemia, respiratory distress and neonatal jaundice seemed to infer an increased risk of autism primarily among term born children. Both respiratory distress and neonatal jaundice are complications which commonly occur among preterm born children[169], and it is possible that the effect of these factors on risk of autism in preterm born children may be concealed by other complications related to preterm birth. Another explanation could be that there is a misclassification among preterm born children due to a vast amount of diagnoses in this group of children. But there may also true differences in risk of autism between preterm and term born children.

There are some general concerns regarding study IV. Several studies have been conducted in Sweden, using register-based data, investigating associations between maternal and/or perinatal factors and later development of autism[118, 178]. Still, no validation of the autism diagnosis in the Inpatient Register has been conducted. We need to assess both the reliability and the representativeness of the autism cases in the Inpatient Register. The rate of infantile autism cases vs. pervasive developmental disorders is often reported to be 1:2-3. In our data, 70% of the cases have infantile autism and the remaining 30% pervasive developmental disorders. This indicates that we have an over-representation of cases with more severe autism. The results should therefore be interpreted cautiously for milder forms of autism. Since the data is abstracted from the Inpatient Register we may have an over-representation of cases with other conditions or diseases rendering in-patient care.

To summarize study IV, we conclude, that factors influencing early brain development may, rather than preterm birth per se, influence the risk of autism. Our study also indicates that there may be differences in risk factors for autism in preterm and term born children.

CONCLUSIONS

Infectious signs in Pap smears can generally not be used to predict preterm delivery. On the other hand if Bacterial vaginosis is detected on Pap smear during the second trimester there is an increased risk of preterm delivery. However, it remains to be determined if the women may benefit from treatment.

Infection with Parvovirus B19 in early pregnancy may be associated with an increased risk of miscarriage or preterm delivery, but larger studies are needed.

Women with previous miscarriage are at increased risk of delivering preterm and the risk increases with severity of preterm delivery. The increased risk of preterm delivery experienced by women with previous miscarriages is confined to preterm delivery with spontaneous onset.

No association could be found between maternal infections and later risk of developing autism. Instead we found that factors directly or indirectly influencing early brain development may, rather than preterm birth per se, influence the risk of autism.

FUTURE RESEARCH

As a result of this thesis, a number of questions with implication for future research have been raised.

In study I we found that presence of Bacterial vaginosis detected in Pap smears within four weeks of delivery was associated with increased risk of preterm delivery before the 32nd week. Most Pap smears were taken in early pregnancy (90% before the 16th week of gestation) and we lack information on whether the women where treated or not. We also lack information on possible confounders or onset of delivery.

In study III we found that previous miscarriage increased the risk of preterm delivery in the subsequent pregnancy and that risk increased with severity of preterm delivery. Further, the association between miscarriage and preterm delivery was limited to preterm deliveries with a spontaneous onset, supporting the hypothesis of a common aetiology of infections in previous miscarriage and preterm delivery. In this study we lacked information on gestational length of miscarriages. Further, previous miscarriage was also only used as a proxy for a woman's susceptibility to infections.

A possible follow-up of these two studies would be conducted by studying whether infectious signs in Pap smear are associated with increased risks of miscarriage. Most Pap smears were taken in early pregnancy and by studying the outcome miscarriage we reduce time between exposure and outcome. Information on miscarriages could be collected from the Inpatient Register since both Uppsala and Gävleborg have full coverage as early as 1964. Further, by adding information on gestational length from medical records we would also be able to study first and second trimester miscarriages separately.

In study II we found no significant association between exposure to viral infection in early pregnancy and risk of miscarriage or preterm delivery. But all point estimates were elevated, indicating a possible association and it would therefore be of interest to repeat the study. The most obvious problem in study II was low power. We also had surprisingly low prevalence of viral infections. We found only one sample positive for CMV, and another study has reported a prevalence of 4% in pregnant women[179]. Also the prevalence of Parvovirus B19 was significantly lower than expected[180, 181]. This could be due to the samples being taken at a single point in early pregnancy, but we may also suspect that we have a number of false negative samples. By developing the analytic methods in a follow-up study power may also increase.

In study IV we found that preterm birth per se did not seem to influence the risk of autism, instead complications during pregnancy and in the neonatal period which could affect brain development increased the risk of autism. A number of complications were associated with increased risk of autism in term born children (hypoglycaemia, neonatal jaundice and respiratory distress). It would be intriguing to further explore these specific exposures and later risk of autism. This may be particularly interesting in term born children since the majority of children who develop autism are born at term. Studies like these could also give more insight into the aetiology of autism. Moreover,

some of these risk factors are actually treatable if detected (neonatal jaundice and hypoglycaemia).

A follow-up on study IV would also have to include a validation of the autism diagnosis in the Inpatient Register. Further, it would be of great interest if the study could be repeated including autism cases from out-patient psychiatric units.

More specifically questions raised from this thesis are:

- Are infectious signs in Pap smear associated with increased risk of miscarriage?
- Does such an association apply to miscarriage in both the first and second trimester?
- Does presence of viral infection in early pregnancy imply an increased risk of miscarriage or preterm delivery?
- Can we find specific, and perhaps treatable, neonatal risk factors for autism?
- Do these risk factors differ between preterm and term born children?
- Do the results from study IV apply to less severe forms of autism?

SVENSK SAMMANFATTNING

Bakgrund

Trots införande av vaccinationsprogram, ökad tillgänglighet av antibiotika och generellt förbättrade levnadsvillkor så är infektionssjukdomar fortfarande en av de vanligaste orsakerna till sjuklighet och död i världen.

Ett växande forskningsfält har under de senast decennierna funnit att infektioner under graviditet ökar risken för både missfall och för tidig förlossning. Men trots att sambanden är tydliga har man inte lyckats minska risken för missfall eller för tidig förlossning.

Sambanden mellan infektion under graviditet och senare missfall eller för tidig förlossning har framförallt observerats med vaginala bakteriella infektioner. Hypotesen är att dessa infektioner vandrar upp genom vagina och livmoderhals för att sedan infektera fosterhinnorna med påföljande inflammation, vilket kan resultera i tidig hinnruptur eller för tidigt värkarbete. Väldigt lite är känt om virusinfektioner under graviditet medför en ökad risk för missfall eller för tidig förlossning.

Missfall och för tidig förlossning ses ofta som oberoende händelser. Det är känt att kvinnor med tidigare missfall löper ökad risk för missfall i nästa graviditet och samma mönster ses med mycket för tidiga förlossningar (innan vecka 32). Eftersom sena missfall (efter vecka 14) och för tidiga förlossningar kan ha en gemensam riskfaktor i infektioner under graviditet, kan man spekulera i huruvida kvinnor med tidigare missfall skulle ha ökad risk även för för tidig förlossning.

Det finns även hypoteser om att infektioner under graviditet skulle öka risken för andra sjukdomar senare i livet. Exempelvis skulle infektioner i nervsystemet under fostertiden eller tidigt i livet kunna påverka den växande hjärnans utveckling. Denna hypotes stöds av studier som visar att barn som drabbas av hjärnhinneinflammation tidigt i livet har ökad risk för psykos och schizofreni som vuxna. Autism är en neuropsykiatrisk störning vars etiologi inte är känd. Virusinfektioner under graviditet kan ha ett samband med ökad risk för autism. Autistiska barn är oftare födda för tidigt och man vet inte om sambandet mellan för tidig förlossning och autism beror på en generell omognad hos det för tidigt födda barnet eller på faktorer som är associerade med för tidig födsel som även ökar risken för autism.

Syfte

Syftet med denna avhandling var att studera infektioner och infektionsrelaterad sjuklighet under graviditet och dess effekter på missfall, för tidig förlossning och autism. Specifikt har vi i studie I studerat om infektionstecken vid gynekologisk cellprovtagning kan användas för att förutsäga risken för för tidig förlossning. I studie II studerade vi om virusinfektioner i tidig graviditet var associerade med ökad risk för missfall eller för tidig förlossning. I den tredje studien undersökte vi om förstföderskor

med tidigare missfall har ökad risk för tidig förlossning. Slutligen studerades om maternella infektioner under graviditet ökar risken för autism hos barnet och om detta samband eventuellt kan förklara den tidigare observerade associationen mellan för tidig födsel och senare risk för autism.

Material och metod

Samtliga studier i denna avhandling är baserade på svenska populationsbaserade kohorter. Delarbete I och III är kohortstudier baserade på registerdata, medan studie II och IV är fall-kontroll-studier. Varje person som föds i eller invandrar till Sverige får ett unikt tiosiffrigt personnummer. Genom att använda personnumret är det möjligt att länka information från olika register till varandra. Detta gör Sverige till ett land med mycket goda förutsättningar för att bedriva epidemiologisk forskning.

I denna avhandling har följande register används:

Medicinska Födelseregistret: Startades 1973 och innehåller information från både mödravårdsjournalen, förlossningsjournalen och den neonatala perioden. I registret ingår data om den gravida kvinnans längd, vikt, rökvanor och eventuella sjukdomar till längd, vikt och eventuell sjuklighet hos det nyfödda barnet. Medicinska födelseregistret täcker mer än 98 % av alla förlossningar i Sverige.

Slutenvårdsregistret: Startades så tidigt som 1964, och har sedan 1987 nationell täckning. Innehåller information om datum för inskrivning och utskrivning samt diagnoser för alla sjukhusinläggningar i Sverige. Diagnoserna är kodade enligt International Classification of Diseases (ICD).

Nationellt Register för Gynekologisk Cellprovtagning: 1969 kallades de första kvinnorna i Sverige till gynekologisk cellprovtagning. Idag har detta program nationell täckning och man rekommenderar idag att alla kvinnor kallas vart tredje (åldrarna 23-50) till vart femte år (åldrarna 51-60). Registret, vars huvudsakliga syfte är tidig upptäckt av cervixcancer, innehåller information om cellfynd, provdatum samt infektions- och inflammationstecken.

Register över blodprover vid Viruslaboratoriet, Karolinska Institutet: Sedan 1975 så ingår screening för Rubella i tidig graviditet i det svenska mödravårdsprogrammet. Idag screenas även för ett antal andra sjukdomar (HIV, syfilis, Hepatit B). Blodprovet, som tas vid något av de första besöken i mödravården sparas sedan enligt de regler som Socialstyrelsen föreskriver.

Medicinska journaler: Även om registren förser oss med stora mängder värdefull information, kan denna ibland behöva kompletteras. I denna avhandling har vi även inhämtat information från medicinska journaler.

Delarbete I

Vi identifierade 60 755 gravida med enkelbörder mellan 1973 och 2000 i Uppsala och Gävleborgs län vilka under graviditeten tagit ett gynekologiskt cellprov. Risken för

antepartal död, för tidig förlossning (innan vecka 37) och lätt för tiden i förhållande till förekomsten av infektionstecken på cellprov beräknades med logistisk regression. Förekomst av Coccobacilli på gynekologiskt cellprov ökade risken för att den nyfödda var lätt för tiden och förekomst av *Trichomonas* ökade risken för måttligt för tidig förlossning (mellan vecka 32 och 36). När endast prover tagna inom fyra veckor innan förlossningen inkluderades så var förekomst av Coccobacilli associerat med en fyrfalt ökad risk för mycket för tidig förlossning (innan vecka 32).

Delarbete II

Bland gravida kvinnor i Stockholms län identifierades 235 fall med missfall i andra trimestern (vecka 14-21), 269 fall med mycket för tidig förlossning (vecka 22-31) och 301 kontroller med förlossning i fullgången tid för vilka vi även hade blodprover tagna i tidig graviditet. Blodproverna analyserades med avseende på förekomst av Parvovirus B19 och Herpesvirus. Virus fanns i blodet hos 11 (4.7 %) kvinnor med missfall i andra trimestern, 10 (3.7 %) kvinnor med mycket för tidig förlossning och 5 (1.7 %) kvinnor med förlossning i fullgången tid, motsvarande oddskvoter (95 % konfidensintervall) på 3.32 (0.93-11.8) respektive 2.21 (0.71-6.84).

Delarbete III

Delarbete III är en kohortstudie baserad på alla förstföderskor med enkelbörder som fött ett levande barn mellan åren 1987 och 2000 i Sverige. Dessa kvinnor länkades till det svenska Slutenvårdsregistret för att få information om tidigare missfall. Med hjälp av logistisk regression beräknades sambandet mellan tidigare missfall och risk för extremt (vecka 22-27), mycket (vecka 28-31) och måttligt (vecka 32-26) för tidig förlossning. Tidigare missfall ökade risken för för tidig förlossning och riskökningen var störst för grupperna med extremt för tidig förlossning och mycket för tidig förlossning. Vidare var tidigare missfall framförallt associerat med en ökning av för tidiga förlossningar som startade med spontan hinnruptur eller spontant värkarbete, medan inget samband fanns med för tidiga förlossningar som startade på annat sätt (vaginalt inducerade, eller planerade kejsarsnitt).

Delarbete IV

Bland barn födda i Sverige mellan 1987 och 2002 identifierades 1216 barn som fick diagnosen autism samt fem gånger så många kontroller (n=6080) matchade för kön, födelseår, förlossningssjukhus. Kontrollen fick heller inte ha fått diagnosen autism vid tidpunkter för fallets insjuknande. Med hjälp av logistisk regression beräknades risken för autism i relation till maternella och graviditetsrelaterade faktorer (inklusive maternella infektioner under graviditet) samt neonatala komplikationer. Jämfört med barn födda i fullgången tid hade för tidigt födda barn en ökad risk för autism: den ojusterade oddskvoten (95 % konfidensintervall) för mycket (vecka 22-31) och måttligt (vecka 32-36) för tidig förlossning var 2.03 (1.13-3.64) respektive 1.52 (1.16-1.99). För att studera den eventuellt medierande effekten av perinatala faktorer på sambandet mellan för tidig förlossning och senare risk för autism så justerade vi först för maternella och graviditetsrelaterade faktorer med resultatet att oddskvoterna sjönk till 1.48 (0.77-2.84) och 1.33 (0.98-1.81) för mycket respektive måttligt för tidiga förlossningar. När vi också justerade för neonatala komplikationer kunde vi inte längre

fastställa några riskökningar mellan mycket eller måttligt förtidig förlossning och senare risk för autism. Riskreduktionerna var framförallt relaterade till preeklampsi, lätt för tiden, missbildningar, lågt Apgar vid fem minuter och neonatala hjärnskador.

Slutsatser

- Förekomst av Coccobacilli (Bakteriell vaginos) på cellprov tagna under andra trimestern var associerat med en ökad risk för mycket för tidig förlossning (innan vecka 32).
- Parvovirus B19 i tidig graviditet kan eventuellt vara associerat med ökad risk för både missfall i andra trimestern och förlossning innan vecka 32. Mer forskning inom området behövs.
- Förstföderskor som tidigare haft missfall har ökad risk att föda för tidigt och risken är störst för extremt (vecka 22-27) och mycket (vecka 28-31) för tidig förlossning.
- För tidigt födda barn har ökad risk att utveckla autism och denna riskökning kan förklaras av maternella, graviditetsrelaterade och neonatala komplikationer som är associerade med för tidig förlossning.

ACKNOWLEDGEMENTS

First of all I would like to thank my supervisor *Sven Cnattingius*. You are not only a great scientist but also a wise man with a big heart and a great sense of humour. Thank you for being my supervisor and not losing patience with my comings and goings at the department. I have learnt immensely from you and I would never have come this far without you.

Secondly, a great thank you to my co-supervisor *Pär Sparén* for always, with a big smile, giving me a helping hand when I showed up with a confused mind and some methodological questions.

Joakim Dillner, my co-supervisor, thank you for your profound knowledge in viral epidemiology.

I then want to start by thanking everyone working at MEB – together you all make this a great place to work! Despite my sometimes long absences of leave you have always made me feel welcome and at home at MEB.

Some persons need special mentioning:

Present and former heads of department *Henrik Grönberg*, *Nancy Pedersen* and *Hans-Olov Adami*, thank you for working so hard to keep the good working spirit at MEB – you do a great job.

A big thanks to all my co-authors: *Stefan Johansson, Anna Johansson, Christina Hultman, Anastasia Illiadou, Sophia Harlid, Malin Sjöholm, Lena Grillner, Mikael Norman*. You have helped me tremendously whenever I asked for it.

Gunilla Sonnebring, besides thorough reading of all manuscript and my thesis and coming with useful points, you always make time whenever I pop by for a chat – it is really appreciated. You are a "rock".

To present and former PhD students: *Lena George, Anna Hübinette, Olof Stephansson, Niklas Bergvall, Stefan Johansson, Jungmei Miao Jonasson, Ylva Trolle Lagerros, Sara Wedrén, Mats Forsman, Sara Öberg, Alexandra Ekman, Louise Eriksson, Therese Moberg, Maria Sandberg, Jurgita Narusyte, Gunnar Larfors, Catarina Jansson, Emma Nilsson, Gabriella Stålberg, Henrik Larsson, Pia Fernberg, Åsa Odenbro, Christina Persson* for everything from scientific discussion to nice lunch chats.

Gunnar Petersson, Ninoa Malki and Mattias Hammarström: thank you for working with the datasets used in the thesis.

For help with all practicalities around my time as a PhD student and dissertation: *Monica Rundgren, Camilla Ahlqvist,* and *Marie Dokken.*

Emma Flordal-Thelander thanks for discussions on all matters from dissertations to pregnancy complications and comforting me in that "Molly the mouse" is good entertainment for children below the age of four.

Thank you for thorough reading of my thesis and questioning my views: *Emma Frans, Fatima Azerkan, Anna-Sofia Melin, Anna Svensson, Ulrika Eriksson and Arvid Sjölander.*

For all lunches and chats on what is really important in life and introducing me to the "afternoon walks": *Ulrika Eriksson* and *Eva Carlström*.

Stefan Johansson, for being my room-mate next door in the "lost" corridor, lots of laughs and discussions on everything from family matters to epidemiology and scientific work.

To present and former colleagues and study friends: *Lena Westman, Miriam Hedin Yangbyn, Agi Supanich, Sofia Rössner*.

To *Peter* and *Emma* for excellent dinners.

To my friends: *Lotta Bjerkö, Eva Blidberg, Anna Nordström, Gabrielle Ekelund, Anna Zethraeus, Candida Savage, Maria Engwall* and *Anna-Karin Ericson* for doing completely different things with your lives and still having so much fun when we get together.

Kris – my mother-in-law. For excellent care of Elvira, it is really appreciated.

Mats, Elin and *Stella* – my brother and family. For being the best brother I could wish for. I hope we get to spend more time together soon.

Pappa and *Eva* – for all support – in my sometimes confused life – things seem to work out in the end!

Mamma – for always saying I could do whatever I wanted.

To the most important persons in my life: *Micke* and *Elvira*, for everything! Without you life would not be the same. I love you! ☺

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