PRETERM BIRTH AND
PRETERM INFANT
A clinical study on certain etiological and diagnostic factors, and the outcome of infants

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Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium 4 of the University Hospital of Oulu, on December 15th, 2000, at 12 noon.

OULUN YLIOPISTO, OULU 2000
Kurkinen-Räty, Merja, Preterm birth and preterm infant
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2000
Oulu, Finland
(Manuscript received: 17 November 2000)

Abstract

The aim of the present study was to evaluate whether bacterial vaginosis (BV) diagnosed in early pregnancy and treated with vaginal clindamycin affects pregnancy outcome, and to investigate the predictive value of interleukins-6 (IL-6) and -8 (IL-8), and insulin-like growth factor-binding protein-1 (IGFBP-1) in cervical secretions, separately and combined by cervical measurement with transvaginal ultrasonography, on preterm delivery. A further aim was to analyze retrospectively the significance of absent or reversed end-diastolic velocity (AREDV) in the umbilical artery on perinatal outcome, and to investigate the short- and long-term outcome of infants born prematurely as a result of various causes (indicated preterm birth, preterm premature rupture of the membranes=PPROM).

Bacterial vaginosis (BV) was screened in 1956 women in a low-risk population at the first antenatal visit, using Gram stain. One hundred and one of 143 BV-positive women were randomized to receive vaginal clindamycin or placebo. Seventy-seven women at 22-32 gestational weeks with premature uterine contractions, and 78 controls were recruited for assay of cervical IL-6, IL-8, and IGFBP-1, and ultrasonographic measurements, which were repeated twice at two-week intervals. Eighty-three women with AREDV in the umbilical artery in high-risk pregnancies at less than 34 gestational weeks (e.g. pre-eclampsia, small-for-gestational age [SGA]) between the years 1988-95 were analyzed retrospectively as regards perinatal outcome. Further, for 103 women between the 24th and the 33rd week of pregnancy, delivered by cesarean section because of maternal or fetal indications, and for 103 matched women, between the years 1990-97, their infants were analyzed as regards neonatal mortality and morbidity, and the outcome at one year of corrected age. Similarly, 78 women with PPROM at gestational weeks 17-30, and 78 controls were also analyzed.

The prevalence of BV was 7.3% (143/1956) and the preterm birth rate in women with BV was 9.9%. Preterm birth occurred in 21% vs. 0% according to whether or not BV persisted. The preterm birth rate was 14% in the clindamycin group vs. 6% in the placebo group. Cervical IL-6 at a concentration of 128 ng/L had a 73% sensitivity and 77% specificity in predicting preterm birth (35% vs. 6%). The combination of IL-6 and a cervical index of >gt 0.2 increased the specificity to 97%, the sensitivity falling to 45%. Concentrations of IGFBP-1 were most elevated (>gt 21 ?g/mL) in cases with neonatal infections (36% vs. 2%). In cases of absent end-diastolic velocity (AEDV) the perinatal mortality (PNM) rate was 9%, compared with 36% in the reversed end-diastolic velocity (REDV) group. Respiratory distress (RDS) and hypoglycemia, and chronic lung disease (CLD; 15% vs. 3%) occurred significantly more often in the indicated than in the spontaneously preterm infants. The PPROM infants had more limb contractures (8% vs. 0%) and pulmonary hypoplasia (12% vs. 5%) and more chronic lung problems up to one year of age than the spontaneously preterm born infants without PPROM.

The persistence of pregnancy BV is a risk factor for preterm birth, but vaginal clindamycin used in a low-risk population in early pregnancy is of no use in reducing the preterm birth rate in cases of BV. The level of IL-6 has a relatively low sensitivity and a limited role as a single method in clinical decision making but in combination with cervical examination by ultrasonography it seems to have a predictive role in cases of threatened preterm birth. A finding of AREDV in the umbilical artery is a warning signal of threatened fetal asphyxia. Infants born after indicated preterm delivery (for fetal or maternal reasons) or PPROM are at risk of later chronic lung disease.

Keywords: ultrasonography, PPROM, bacterial vaginosis, interleukins, insulin-like growth factor-binding protein-1.
To fetal and infant life
Acknowledgements

The research for this thesis was carried out at the Departments of Obstetrics and Gynecology and Pediatrics, Oulu University, during the years 1995–2000.

I wish to express my most sincere gratitude to my supervisor, Professor Pentti Jouppila, M.D., Head of the Department of Obstetrics and Gynecology. His firm hold and experience both as a scientist and a clinician encouraged me to finish this work. He always had a moment in the middle of professorial tasks to guide me through difficult as well as joyful moments.

I owe my sincere thanks to Emeritus Professor Antti Kauppila, M.D., who provided me the opportunity to undertake my specialization in the field of Obstetrics and Gynecology.

I am most deeply indebted to my second supervisor, Docent Maila Koivisto, M.D., who suggested the neonatological part of this study and guided me in numerous scrupulous discussions with a warm attitude. This part of the study gave me as an obstetrician much more to think about concerning the later events after the perinatal period.

I would like to express my gratitude to Docent Ulla Ekblad, M.D., and Docent Outi Tammela, M.D., who kindly agreed to be the official examiners of this thesis. Their critical appraisal and constructive comments have been invaluable for me.

I warmly thank Professor Juha Tapanainen, M.D., for his scientific criticism during the last year of my specialization in the gynecological endocrinology unit. I owe a great debt of gratitude to Docent Anna-Liisa Hartikainen for her experienced epidemiological advice – her example aroused my interest in perinatology. I would like to express my gratitude to Docent Tapio Ranta, my chief at Päijät-Häme Central Hospital. He helped in the arrangement of my daily work, giving me time to write this thesis, and his positive attitude made it possible for me to finish of this work. I want to thank Professor Jorma Paavonen and Docent Tapio Kurki for giving me the opportunity to collaborate with them. I thank Salme Vuopala, M.D., for demonstrations in the field of cervical cytology. My warmest thanks go to Professor Aimo Ruokonen and Docent Eeva-Marja Rutanen, who always had time for consultation and who enlightened me when there were problems in clinical chemistry. I would like to express my thanks to Markku Koskela, M.D., whose personnel in the microbiology laboratory did an enormous amount of work with the gram stains, and special thanks go to Mrs. Seija Liukkonen.
I wish to thank Aarre Kivelä for first showing me the “enter” button on the computer and later Leo Mäkäräinen for introducing me to ultrasonographic equipment. I owe deepest thanks to Docent Aydin Tekay and Candido Tomas, M.D., Ph.D, who introduced me to audiovisual technology for congress materials. My colleague Jari Johansson, M.D., has given me technical and psychological assistance with computer problems, despite the long distance, for which I am deeply grateful. I also want to thank my colleague Ilkka Järvelä for numerous consultations concerning computer programs, and Jukka Olanterä at Päijät-Häme Central Hospital, for the same. I want to express my gratitude to my colleague Riitta Koivunen for her unselfish and extraordinary help and great friendship and with whom I managed to do this work sometimes at the hours of night. I also wish to thank my colleagues Laure Morin-Papunen, Kaarin Mäkikallio-Anttila, Heli Spalding, Eila Suvanto-Luukkonen, Juha Räsänen, Anni Perheentupa, Anneli Pouta, Ainokaisa Karaiste and Tiina Posa and many others for their never-ending support.

I express my gratitude to Michael Spalding, M.D., and Nick Bolton, Ph.D., for their help in revising the English language. Sirkka Pramila, M.Sc., will always be in my memories with great gratitude and with a deep feeling of loss, for her modest attitude and diligent work in the field of statistics. Sadly, she passed away last autumn. Risto Bloigu, M.Sc., is owed a great debt for helping me with statistical problems in spite of the geographical distance. I also thank my current colleagues in the Department of Obstet Gynecol at Helsinki University, who have with a friendly and inspiring spirit given me great support during my ongoing specialization in perinatology.

I am grateful to all the midwives in Oulu district outpatient clinics for participating in the recruitment of subjects for my studies and for the enormous amount of screening work they have done. Warmest thanks go to all the women of Oulu taking part in my study.

I also wish to express my gratitude to Ritva Vasala and Pirjo Ylimartimo for their assistance in providing the examination facilities and for their enormous help in specimen storage and transportation problems, and to Anneli Tiittanen and Maira Syväri for their never-ending secretarial help, thanks which also go to the secretary of Päijät-Häme Central Hospital, Anja Niinivirta, and librarian Airi Kokko. I am thankful to Mrs. Liisa Kärki of photography laboratory for providing slides and illustrations for articles. I am also grateful to Mrs. Kaija Salkosalo for the thousands of documents which she provided from the archives.

Finally, my warmest thanks belong to my beloved husband Timo. He encouraged me to start this work and has always supported me with an unselfish attitude. Despite the long distance and the short weekends and ever shorter summer holidays, he stayed beside me. I also want to express my gratitude to my brother Kari for his understanding during these years.

This research has been supported financially by research grants from Oulu and Helsinki Universities, the Research Foundation of Oulu University, the Research Foundation of Obstetric and Gynecology, the Finnish Perinatal Society, Helsinki University Support Foundation, and by Pharmacia Upjohn, and Medix Biochemica.

Lahti, December 2000
Abbreviations

AEDV  absent end-diastolic velocity
AF  amniotic fluid
AREDV  absent or reversed end-diastolic velocity
BPD  bronchopulmonary dysplasia
BPS  biophysical scoring
BV  bacterial vaginosis
CI  confidence interval
CLD  chronic lung disease
CP  cerebral palsy
CRP  C-reactive protein
CS  cesarean section
CTG  cardiotocography
ELBW  extremely low birth weight
EPO  erythropoietin
FHR  fetal heart rate
FiO2  fraction of inhaled oxygen
FIRS  fetal inflammatory response syndrome
FVW  flow velocity waveform
GA  gestational age
GBS  group B streptococcus
HELLP  hemolysis, elevated liver enzymes, low platelets
ICD  International Classification of Diseases
IGFBP-1  insulin-like growth factor-binding protein-1
IL  interleukin
IUGR  intrauterine growth restriction
IVH  intraventricular hemorrhagia
LBW  low birth weight
MIAC  microbial invasion of the amniotic cavity
NICU  neonatal intensive care unit
NNM  neonatal mortality
NPV  negative predictive value
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<td>PI</td>
<td>pulsatility index</td>
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<td>PNM</td>
<td>perinatal mortality</td>
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<td>PPROM</td>
<td>preterm premature rupture of membranes</td>
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<td>PPV</td>
<td>positive predictive value</td>
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<td>PROM</td>
<td>preterm rupture of membranes</td>
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<td>PVL</td>
<td>periventricular leukomalacia</td>
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<td>RDS</td>
<td>respiratory distress syndrome</td>
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<td>REDV</td>
<td>reversed end-diastolic velocity</td>
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<tr>
<td>RI</td>
<td>resistance index</td>
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<td>ROC</td>
<td>receiver operator characteristic</td>
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<td>RR</td>
<td>relative risk</td>
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<td>S/D</td>
<td>systolic-diastolic ratio</td>
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<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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<tr>
<td>SGA</td>
<td>small for gestational age</td>
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<td>VLBW</td>
<td>very low birth weight</td>
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<td>WHO</td>
<td>World Health Organization</td>
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List of original articles

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1 Introduction

Preterm birth referring to birth that occurs at a gestational age of less than 37 completed weeks (nomenclature of the World Health Organization [WHO]), is still one of the major problems in peri- and neonatology. The incidence of preterm birth varies between 5% and 10% in Western countries, but figures as high as 17% are reported among lower social class populations (Papiernik et al. 1985). The proportion of low birth weight (LBW) (<2500 g) infants was 4.4% in 1998 and it has remained relatively constant in Finland, but in the nineties it increased somewhat (Koskinen et al. 1999). The same trend is seen in Canada, where the rate of preterm births rose from 6.3% to 6.8% in a ten-year period. The reason for this has been attributed to increased rate of multiple births, an increased rate of obstetric intervention, the use of ultrasound in gestational age estimates and changes in statistical reporting (Joseph et al. 1998).

The main cause of perinatal mortality (PNM) is the morbidity associated with preterm delivery of the infant. Eighty-five percent of early neonatal deaths (<7 days of life) that are not caused by congenital malformations are due to preterm birth (Berkowitz & Papiernik 1993, Koskinen et al. 1999). Although improved care has resulted in a decline in PNM, morbidity is still high (Lee et al. 1995, Hack & Fanaroff 1999, Wood et al. 2000). The morbidity caused by prematurity extends to later life, resulting in enormous psychological and economic costs.

Epidemiological risks factors have been evaluated in cases of idiopathic preterm delivery, preterm premature rupture of membranes (PPROM) and indicated preterm birth (Savitz et al. 1991). The most important risk factors seem to be previous preterm birth and antepartum bleeding. The diverse etiology of preterm birth makes its prediction and thus any intervention difficult. Infections – often subclinical – seem to play a part in a third of preterm deliveries (Lamont & Fisk 1993, Keirse 1995).

Therapeutic regimens to prevent preterm birth are limited in efficacy. New drugs, such as oxytocin antagonists, have been tested in trials, but their clinical efficacy is limited (Shubert 1995). With the aid of a suitable screening test, candidates for closer intervention could be selected. Needless hospital admissions could thus also be avoided. Resources should be targeted towards preventing the problem, in otherwords, identification of women at risk would be a key to the prevention of preterm delivery. No sufficiently specific marker, however, has so far been found.
In cases of indicated prematurity (such as pre-eclampsia and intrauterine growth retardation [IUGR]) an obstetrician has to decide the optimal time for delivery while recognizing the risks of prematurity, on the other hand, threatened intrauterine fetal asphyxia. Antenatal determination of fetal asphyxia is, however, difficult despite various monitoring methods. Doppler ultrasonography has provided us with a tool for fetal surveillance in high risk pregnancies, and it has proven to be an evidence-based method in diminishing PNM (Bricker & Neilson 2000).

The neonatal outcome of preterm infants has been evaluated in numerous studies, but the long-term consequences in certain specific obstetric groups have been more sparsely evaluated (Spinillo et al. 1995). Previous studies mainly concern the neurologic outcome, and the late ramifications in regards to pulmonary development have been evaluated rarely (Tammela & Koivisto 1992, Nelson et al. 1994). In PPROM cases, for example, there can be associated oligohydramnios causing pulmonary hypoplasia (Nimrod et al. 1984). Recently, the term fetal inflammatory response syndrome (FIRS) has been introduced (Gomez et al. 1998), and it has been shown to be a risk factor for severe neonatal morbidity in the forms of respiratory problems which also affect central nervous system.

The present clinical study was performed in order to evaluate the significance of bacterial vaginosis and its treatment with vaginal clindamycin on preterm birth, and whether different markers or a combination of different methods – biochemical and ultrasonographic – can improve the prediction of preterm delivery. Finally, the hypothesis was proposed that in addition to neonatal morbidity, the long-term pulmonary outcome of infants born prematurely as a result of diverse etiological causes maybe different.
2 Review of the literature

2.1 Preterm birth

2.1.1 Definitions

Until ultrasonography came into use, gestational age was based on the last menstrual period. Hence the concept of prematurity was obscure until Ylppö suggested (Ylppö 1913) as a birth weight under 2500 g a limit of prematurity. According to the WHO definition (WHO 1980), a delivery is preterm when occurring before the 37th completed week of pregnancy. Even today the statistics, however, vary greatly between different countries, and therefore it is difficult to compare the rates of prematurity all over the world. According to the nomenclature of the International Classification of Diseases (ICD-9), a weight limit to birth is 500 g and/or 22 gestational weeks. ICD-9 defines infants according to birth weight: low (LBW = under 2500 g), very low (VLBW = under 1500 g) and extremely low birth weight (ELBW = under 1000 g). The term IUGR is usually defined as birth weight for gestational age below the 10th percentile of the reference standard. Another definition includes infants whose birth weight is two or more standard deviations below the mean value for gestational age (Karlberg et al. 1985).

By PNM is meant the proportion of stillborn infants and infants dying during the first week (under 7 days) of life per thousand births. Neonatal mortality (NNM) concerns infants dying at less than 28 days of life per thousand liveborn infants. Infant mortality concerns infants dying in the first year of life. Maternal mortality per 100 000 is defined as a woman’s death during pregnancy or within 42 days after the end of pregnancy independent of the duration or nature of pregnancy (causes include those connected to pregnancy or worsened by pregnancy).
2.1.2 Incidence

The incidence of preterm birth (< 37 weeks) varies greatly in different populations, being on average between 4–15% (Papiernik et al. 1985). There are racial differences in the preterm birth rate, being highest among the black population in the United States (Savitz et al. 1991). Rantakallio et al. showed a decline of 4% in the preterm birth rate in a 20-year period (1966-1986) from 9% to 5% in Northern Finland (Rantakallio et al. 1991). The reason for this trend remains somewhat obscure, although an increased standard of living is claimed to cause a fall in the preterm birth rate. In contrast, in Northern England, a rise in the incidence of preterm birth was noticed between 1983 and 1994 (Tin et al. 1997), and a similar trend has also recently been seen in Finland (Koskinen et al. 1999).

It has been speculated that an increased rate of indicated (= iatrogenic) preterm delivery is one of the causes of this increase. Nearly one third of preterm births are indicated preterm (Meis et al. 1998), and even higher frequencies (38%) of indicated preterm birth have been reported (Kimberlin et al. 1999). These are mainly due to hypertensive disorders of pregnancy. It is possible that an older age of primiparas exposes them to the risk of already having a chronic disease. An increased rate of cesarean sections (CS) has also been seen in the context of rising preterm birth rates (Agdestein 1994). In addition, gestational age determinations based on ultrasonography, an increase in multiple births and definitions in statistical reporting play a role. In Finland the incidence of preterm birth was 5.9% and that of LBW infants 4.4% in 1998 according to the latest national statistics (Koskinen et al. 1999). In general, the spontaneous preterm birth rate has remained relatively constant with the exceptions of the increase in multiple pregnancies by means of artificial reproduction technologies, and thus increased risk of prematurity is mainly due to an increase in indicated preterm delivery.

2.1.3 Risk factors

Certain risk factors for preterm birth have been recognized in epidemiological studies (Berkowitz & Papiernik 1993). These can be categorized to sociodemographic (maternal age, race/ethnicity, marital status, socioeconomic status and psychosocial factors), genetic, constitutional and nutritional factors. In addition obstetric history and medical complications of pregnancy play a marked role. PPROM and infections are reviewed in detail below.

Age over 35 has been found to be a risk factor for preterm birth (Wen et al. 1990) and this is thought to be due to an increase in maternal chronic disease with advancing age, e.g. essential hypertension (Ekblad & Vilpa 1994). Young age has also been found to be a risk factor for prematurity. Adolescents may use prenatal care inadequately and more often belong to a lower social class than their adult counterparts. In multivariate analysis, however, these differences are not so clear. Gynecologic age (under 2 years between menarche and pregnancy) is a risk factor for prematurity rather than chronologic young age, when analysing very young (12–15 years of age) primiparas (Scholl et al. 1992). In that study multiparas aged below 19 years had no increased risk of preterm birth.
compared with older multiparas. According to another study older primiparas were not found to have a higher preterm birth rate compared with younger ones (Berkowitz et al. 1990). The inconsistencies between the results may be partly due to sample sizes or different populations studied.

Racial differences seem to be clear according to all studies, with black women having higher preterm birth rates (Shiono & Klebanoff 1986). The gestational age among black women appears to be shifted one week earlier (Papiernik et al. 1990). The NNM rate is lower for blacks than for whites before 37 weeks, but higher at 37 weeks and later (Sappenfield et al. 1987). Socioeconomic status may, of course, be a confounding factor.

Lower social class has been found to be a risk factor for prematurity in many studies (Kaminski et al. 1973, Berkowitz 1981, Rantakallio & Oja 1990). Socioeconomic status is often a mirror of other demographic features, such as marital, educational and professional status. Hence it cannot easily be controlled in epidemiological studies.

Low maternal weight gain (Wen et al. 1990, Kramer et al. 1992) is positively correlated to preterm birth rate. It has, however, been claimed that low maternal weight gain is possibly a symptom of a small infant (Kramer et al. 1992) because this association disappear when infant birth weight is subtracted from total weight gain. Interpreting such results may be difficult because of the difference in body mass and fluid retention. Anemia has been suggested to be as risk factor for preterm birth (Lieberman et al. 1987, Klebanoff et al. 1991).

Smoking is a risk factor for IUGR, but it is also associated with an increased preterm birth rate (Hartikainen-Sorri & Sorri 1989), although not in all studies (Sipila et al. 1992). Cigarette smoking is also related to PPROM (Shiono et al. 1986). As regards drug use, which is devastatingly increasing, cocaine is clearly correlated to preterm birth and placental abruption (Chasnoff 1991). Alcohol consumption is unlikely to be a independent risk factor of preterm birth (Wen et al. 1990).

The role of physical activity is controversial. Industrial work and mentally unstimulating tasks have been reported associated with preterm birth. Strenuous work itself may not always be worst, in comparison with psychological stress. It has been found that work leave for fatigue – without medical reasons – decreased preterm birth rates (Mamelle et al. 1984). Not all studies, however, have shown the same findings. Employment characteristics have not been found to be associated with a risk of preterm birth (Hartikainen-Sorri & Sorri 1989). Exercise during pregnancy might even reduce the risk for preterm birth (Berkowitz et al. 1983). Sexual activity during pregnancy has no overall association with preterm birth, but in certain subgroups, infections connected to intercourse may be the reason for increased preterm birth rates (Klebanoff et al. 1984).

Chronic maternal disorders, such as cardiopulmonary, diabetes, asthma, epilepsy and hypothyroidism, are connected to preterm birth, but it has to be noted that these disorders may be associated with an increased rate of medical intervention – cesarean sections or labor induction – leading to a recorded preterm birth.

Multiple gestation increase the risk of preterm labor and birth. Mechanical distention of the uterus and maybe hormonal excess are underlying mechanisms (Guzick 1984). This is important while the rates of multiple pregnancies are becoming higher due to artificial reproduction technologies.

First and subsequent trimester bleeding is connected to an increased risk of preterm birth (Williams et al. 1991). Harger (Harger et al. 1990) found bleeding in more than one
trimester to be a risk factor of PPROM. Histopathological hemosiderin deposits are found in premature placentas, which is evidence of decidual hemorrhage (Salafia et al. 1995).

Obstetric history has a major effect on the risk of preterm birth. Previous preterm or LBW delivery is an evident risk for preterm birth (Kaminski et al. 1973, Berkowitz 1981, Mamelle et al. 1984). It is also a risk for PPROM (Harger et al. 1990). Previous spontaneous abortions are not important in preterm birth risk assessment (Kramer 1987). Maybe first trimester bleeding that does not lead to abortion, leads to choriodecidual damage that makes a mother prone to preterm delivery in later trimesters. Prior induced abortions are not clearly associated with the risk of preterm birth (Shiono & Klebanoff 1986). The reason maybe that current techniques with vacuum aspiration or even medical treatment are relatively atraumatic. Infertility might increase preterm birth rates because of treatment procedures, use of gonadotropins, increased rate of multiple pregnancies, or the underlying cause of infertility (Berkowitz 1981, Doyle et al. 1992). It can be concluded from the literature that the most important obstetric risk factor of preterm birth is previous preterm birth.

2.1.3.1 Preterm premature rupture of membranes (PPROM)

Preterm rupture of membranes (PROM) is defined as premature rupture of membranes before the onset of labor at any stage of gestation. Preterm premature rupture of membranes (PPROM) is defined as PROM occurring before 37 weeks of completed pregnancy (Romero et al. 1999). The incidence of PROM is reported to be 10% of deliveries after 37 weeks of gestation (Fayez et al. 1978, Daikoku et al. 1982). PPROM complicates 2–3% of all pregnancies and is associated with 30% of all preterm births (Arias & Tomich 1982). The latent period describes the time from rupture of membranes to the onset of labor contractions (Belady et al. 1997). About 80% of women with term PROM go into delivery within 24 hours (Gunn et al. 1970). Of the patients with PPROM before 34 weeks of gestation, 60% have been reported to go into labor within 48 hours (Cox et al. 1988). The latent period is inversely correlated to gestational age.

Epidemiological risk factors for PPROM are smoking, previous preterm delivery and bleeding during pregnancy (Harger et al. 1990). Historical biophysical studies have involved determination of the tensile strength of membranes and its potential role in PPROM (Duncan 1869). Recent biochemical studies on collagen and proteolytic activity have focused on matrix metalloproteinases (Vadillo-Ortega et al. 1990). Choriodecidual damage seems to be the factor exposing mother and child to the risk of preterm delivery. Alterations in trophoblast invasion caused by biochemical factors may lead to PROM.

An important etiological factor of PPROM according to recent knowledge appears to be an underlying infection (Naeye & Peters 1980). Membrane degradation is stimulated by microbial invasion (Escherichia coli, GBS) (Sbarra et al. 1987). One of the microbes associated with PPROM is chlamydia (Gravett et al. 1986). Evidence of an ascending infection has been clearly documented (Carroll et al. 1996). In 76% of the cases the same flora were retrieved from the amniotic cavity, vagina and endocervix. Microbial invasion of the amniotic cavity (MIAC) has been shown in PPROM in several studies, confirmed by at high rate of positive amniotic fluid cultures (30–50%) (Romero et al. 1988). The
most frequent isolates from amniotic fluid (AF) are *Ureaplasma urealyticum* and *Mycoplasma hominis*, followed by GBS, *Fusobacterium* and *Gardnerella vaginalis* (Carroll et al. 1996). MIAC can also be a consequence of PPROM (Naeye & Peters 1980), and pregnancy prolongation has been reported in PPROM cases with the use of antibiotics (Mercer & Arheart 1995). Inflammatory mediators, cytokines, are implicated in PPROM. Interleukin-1 increases prostaglandin synthesis (Romero et al. 1989). Interleukin-6 has been detected in the amniotic fluid in cases of PPROM and infection (Romero et al. 1990).

Recently, fetal inflammatory response syndrome (FIRS) has been defined as elevated IL-6 concentrations in fetal plasma (Gomez et al. 1998). These fetuses have impending onset of delivery regardless of the microbial status of the AF.

Complications of PPROM on the maternal side include increased rates of CS and endometritis (Major & Kitzmiller 1990). Severe complications include cord prolapse, abruptio placentae and fetal distress. The neonatal complications are related to prematurity and consist mainly of respiratory problems, intraventricular hemorrhage (IVH) and infections (Beydoun & Yasin 1986, Moretti & Sibai 1988, Morales & Talley 1993, Nelson et al. 1994). Nimrod et al. reported pulmonary hypoplasia and orthopedic deformities associated with PPROM (Nimrod et al. 1984).

The diagnosis of PPROM is sometimes clear, but a nitrazine test (Friedman & McElin 1969), or a vaginal test of IGFBP-1 (Rutanen et al. 1993), or a ferning pattern of amniotic fluid (Reece et al. 1984) can be used in uncertain cases. A diminished amount of AF as observed ultrasonographically can also support the finding. Digital examination is discouraged (ACOG 1998). Leukocyte count and C-reactive protein are used as markers of imminent systemic infection (Kurki et al. 1990) and local microbial pathogens screened and treated (ACOG 1998, Ohlsson & Wang 1990).

Treatment depends on gestational age: over 34 weeks, the main recommendation is that delivery should be induced. Expectant management of PPROM is used in very early cases, but general protocols are difficult to establish. Antimicrobial prophylaxis is nowadays recommended (Egarter et al. 1996). Antimicrobial treatment seems to reduce IVH and neonatal sepsis (Mercer & Arheart 1995). The optimal antibiotic, route of administration and duration of treatment are, however, still controversial.

Corticosteroids should be given in the absence of an evident infection. The use of tocolysis is of little advantage, but at least provides time for corticoids to act (ACOG 1998). The early weeks of gestation and PPROM are very problematic because of the impending risk of fetal pulmonary hypoplasia with potentially severe consequences in the developing lung.

### 2.1.3.2 Infections

*Chlamydia trachomatis* is a well known risk factor as regards adverse pregnancy outcome (Hardy et al. 1984, Paavonen & Eggert-Kruse 1999) and an association with preterm delivery and spontaneous abortion has been observed. No clear evidence of *Neisseria gonorrhoeae* increasing the risk of prematurity has been found. Asymptomatic bacteriuria is present in 2–10% of pregnant women, and it should be treated because of the risk of
increased preterm birth and pyelonephritis of the mother (Smaill 1998). Screening programs for vaginal GBS infections, which are relatively common (15–40%) (Schuchat 1998), are recommended in the USA, aimed at preventing the potentially lethal condition - neonatal sepsis (Brozanski et al. 2000).

The finding that bacterial vaginosis (BV) increases the risk of preterm birth and PPROM has led to numerous studies and screening and treatment protocols concerning BV (Kurki et al. 1992). Its association with adverse pregnancy outcome is documented in the form of preterm birth, PPROM, chorioamnionitis and postpartum endometritis (Gravett et al. 1986, Kurki et al. 1992, Meis et al. 1995, Riduan et al. 1993). Bacterial vaginosis is characterized as a change in the microbial balance of vaginal flora (Kimberlin & Andrews 1998). The condition is marked by a clear increase in the concentrations of *Gardnerella vaginalis* (Döderlein 1894, Gardner & Dukes 1955) *Mycoplasma hominis*, and anaerobes including *Bacteroides* and *Mobiluncus* species, with the absence of lactobacilli (Eschenbach et al. 1988, Hillier 1993). This altered flora exists as a continuum ranging from normal to intermediate and to BV. It is characterized by a grayish, homogeneous vaginal discharge.

Bacterial vaginosis is reported to occur in 15–20% of pregnant women (Hillier et al. 1992, Eschenbach 1993). It can be a relatively symptomless disorder, with 20–50% of pregnant women being unaware of having BV (Amsel et al. 1983).

The diagnosis of BV (Amsel et al. 1983) is based on three of the following criteria: vaginal pH > 4.5, positive amine odor with 10% potassium hydroxide, clue cells (Fig. 1) (epithelial cells with adherent bacteria) and a typical vaginal discharge. A Gram stain for diagnosis of BV was introduced by Spiegel (Spiegel et al. 1983) and modified by Nugent (Nugent et al. 1991). Culture is not a reliable means of diagnosing BV. Pap smears also seem to be insufficient to screen for BV during pregnancy (Greene et al. 2000).

![Fig. 1. A clue cell indicating bacterial vaginosis.](image)

According to the present evidence, treatment of BV is useful at least in high-risk pregnancies (e.g. women with a history of previous preterm birth or a low pre-pregnancy
weight) as regards reducing the preterm birth rate (Morales et al. 1994, Hauth et al. 1995, ACOG 1998). Systemic or local antimicrobial drugs may be used. Vaginal clindamycin has been shown not to reduce the preterm birth rate in a low-risk Indonesian population, compared with placebo (Joesoef et al. 1995). A high spontaneous resolution rate (55%) is speculated to be a explanation for this, and randomization perhaps occurred too late (at 14 to 26 weeks). Early invasion of BV to the higher genital tract may lead to a later risk of preterm birth (Hillier et al. 1988). Vaginal clindamycin was effective in treating BV in a study by McGregor et al. (McGregor et al. 1994). Local treatment with clindamycin was found, on the other hand, to be ineffective in a high-risk population, but the medication was applied later (up to 32 weeks) (Vermeulen & Bruinse 1999). In a subgroup analysis of a high-risk population, oral metronidazole treatment at 24 weeks appeared to be effective in reducing the preterm birth rate (McDonald et al. 1997). The matter of possible re-treatment is unclear. The spontaneous resolution rate of BV has been evaluated (Hay et al. 1994), and in 50% of cases normal lactobacillus flora is regenerated. So far, results dealing with the efficacy of BV treatment during pregnancy are controversial.

2.2 Indicated preterm birth

The rates of indicated preterm birth have shown an increasing tendency in recent years (Piekkala et al. 1986, Olsen et al. 1995). The indication can be maternal or fetal, most common being hypertensive disorder of pregnancy, and threatened fetal asphyxia (Ekbland & Vilkka 1994). Indicated and spontaneous preterm births have different risk profiles (Meis et al. 1995). Indicated preterm birth is associated with older maternal age, mothers with previous stillbirth and early pregnancy bleeding, whereas spontaneous preterm births are typical in younger mothers, smokers and low weight mothers. Berkowitz found variations in risk factors in a population of over 31 000 women, hypertension and diabetes increasing the risk of indicated preterm birth to 8- and 9-fold (Berkowitz et al. 1998).

A recent report from the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network showed, however, no differences in neonatal outcome in infants born at < 1000 g for various reasons (Kimberlin et al. 1999). Univariate parameters suggested lower IVH rates among indicated preterm infants than in spontaneous ones at one year of age. It is commonly thought that a “stressed” environment enhances fetal adaptation to neonatal life. The immediate neonatal outcome, however, did not differ according to reason for preterm birth, in one study (Iannucci et al. 1996), nor did later survival according to another study (Wolf et al. 1993). In addition, Owen et al. (Owen et al. 1990) showed no perinatal survival advantage in cases of indicated preterm birth.

The long-term outcome of these infants has also been studied (Spinillo et al. 1994), and interest has concentrated on neurologic outcome. Certain etiological subgroups of prematurity, however, have only sparsely been evaluated.
2.2.1 Maternal hypertensive disorders and placental insufficiency

Placental insufficiency is caused by reduced maternal blood flow to the fetoplacental unit. The placental blood supply is established extravillous trophoblast invading the spiral arteries in the placental bed. This system has a partial failure in pre-eclampsia. The factors controlling the mechanisms are unclear, but two components have been described in the failure: in pre-eclampsia some vessels fail to change. In the second stage, endovascular trophoblast fails to advance into the intramyometrial part of these vessels, and hence there is incomplete transformation of the spiral arteries into low-resistance uteroplacental vessels (Robertson et al. 1975). Sometimes a similar situation is found in nonhypertensive pregnancies, where ischemic damage of the placenta is seen histopathologically (van der Veen & Fox 1983). Hemodynamic studies have demonstrated, however, reduced blood flow in both situations (Ferrazzi et al. 1994).

Placental circulation normally has a uniquely low resistance, with 50% of the combined cardiac output of the lamb fetus going into the umbilical circulation (Rudolph 1985), compared with the 25% of the combined human ventricular output through high-resistance fetal lungs (Rässänen et al. 1996).

Hypertensive diseases complicate roughly 10% of pregnancies, depending on the criteria and population. Pre-eclampsia accounts for 70% of cases of hypertension in pregnancy; essential hypertension the remaining 30% (Erkkola 1989). The term gestational hypertension is used when a previously healthy woman develops hypertension (140 mmHg systolic and 90 mmHg diastolic or greater) after 20 weeks of pregnancy (ACOG 1996). This can be a manifestation of future pre-eclampsia or unrecognized chronic hypertension. Proteinuria over 0.3 g is considered clinically significant. Edema is subjective, but when generalized it should be considered pathological. The criteria of severe pre-eclampsia are blood pressure over 160 mmHg systolic or over 110 mmHg diastolic, and daily proteinuria over 5 g (in the severest forms oliguria). Maternal symptoms include headache, visual disturbances, epigastric pain, and even seizures. Eclampsia is the occurrence of seizures or coma. Hemolysis, elevated liver enzyme concentrations and low platelet count are together called HELLP syndrome (Weinstein 1982). Superimposed pre-eclampsia is defined as pre-eclampsia developing in a woman having chronic hypertension. This occurs in 15–30% of women with this disorder. If pre-eclampsia is manifest in early pregnancy, it can be a sign of underlying renal disease (Ihle et al. 1987). Also metabolic disorders, such as hyperinsulinemia, can be seen later in life in women with pre-eclampsia (Laivuori et al. 1996).

Risk factors include nulliparity, multiple gestation, family history, previous history of pre-eclampsia, diabetes, molar pregnancy, fetal chromosomal aberrations and nonimmune hydrops fetalis (Sibai & Moretti 1988). The aforementioned spiral arterial changes are, however, secondary phenomena. The etiology is still unknown. Possibly, genetic or immunological mechanisms are also involved (Witlin & Sibai 1999).

Placental malfunction causes fetal chronic asphyxia and IUGR. Placental insufficiency and preterm birth may share some common etiological factors according to histopathological and Doppler velocimetry findings. Stringini et al. found that the uterine artery systolic/diastolic (S/D) at 25–36 weeks of gestation was elevated in women who delivered preterm. The finding was consistent whether the fetus was SGA or non-SGA.
Histologic fibrous lesions other than those characteristic of infection have been detected in placentas of prematurely born infants (Salafia et al. 1991, Arias et al. 1993).

### 2.3 Diagnostic tests

#### 2.3.1 Biochemical markers

There are several biochemical markers reflecting the inflammatory condition of chorioamnion and cervical tissue. The first and perhaps the most studied cervical inflammatory mediator is fetal fibronectin (Lockwood et al. 1991). This is an extracellular matrix constituent in the maternal-fetal interface, and it can be detected in cervicovaginal secretions. Due to its relatively low sensitivity (Chien et al. 1997), its assay has not reached overall acceptance in clinical decision-making concerning threatened premature delivery, though it can be used to select subjects for closer follow up. Its short-term (7-day) predictive value of birth below 34 weeks of gestation seems, however, to be good (Leitich et al. 1999). It can identify subjects at risk of preterm birth with over 80% sensitivity and specificity, but that does not necessarily result in improved pregnancy outcomes (Lockwood et al. 1991).

The role of cytokines, to which, for example, interleukins belong, in the etiology of preterm birth has aroused the interest of scientific researchers since the eighties (Mitchell et al. 1991, Mitchell et al. 1993). These soluble mediators are part of the communication system between cells (Mitchell et al. 1993). At least sixteen different types of interleukin are characterized (Athayde et al. 2000). The most studied in human trophoblastic tissues are IL-1, tumor necrosis factor-alfa (TNF-alfa), IL-6 and IL-8. Bacterial products induce cytokine synthesis and activation of prostaglandins. Increased production of prostaglandins seems to be a consequence of increased concentrations of IL-1, IL-6, IL-8 and TNF-alfa (Romero et al. 1989, Romero et al. 1989, Romero et al. 1990, Keelan et al. 2000). Amniotic fluid in intra-amniotic infection had greater concentrations of these cytokines than AF from women without infection. Interleukin-1 is present in the amniotic cavity in the third trimester, TNF-alfa in the second and third trimester, and IL-6 in the second trimester onwards (Mitchell et al. 1993). Decidual tissue is a source of these cytokines, but monocytes, lymphocytes and endothelial cells also produce them (Dudley et al. 1992, Dudley et al. 1993). These cytokines can also be isolated from cervical fluids (Tanaka et al. 1999). By measurement of cervical IL-6 it is possible to identify intra-amniotic infection among pregnancies with preterm labor and intact membranes (Rizzo et al. 1996). Elevated serum IL-6 concentrations have been found to be positively correlated with preterm birth (Murtha et al. 1998). Relatively low sensitivity has hindered the clinical applicability of these tests so far.

Insulin-like growth factor-binding protein-1 has been proposed to be an endometrial and decidual molecule (Seppälä et al. 1994). It is, one of the major secretory proteins of decidualized endometrium and was formerly known as placental protein 12 (Giudice 1997). Members of the IGF-system include IGF peptides, IGF-binding proteins (IGFBPs
1-6) and IGF receptors and proteases (Giudice & Irwin 1999). IGFBPs modulate the anabolic and insulin-like effects of the IGFs. IGFBP-1 was isolated 15 years ago, and a radioimmunoassay was developed measuring both phosphorylated and dephosphorylated IGFBP-1 isoforms (Brismar et al. 1995). The amnion mainly contains nonphosphorylated isoforms, as do fetal serum and maternal plasma, but the phosphorylated isoform predominates in decidual cells and liver (Westwood et al. 1994).

In cases of preterm rupture of membranes, IGFBP-1 is found in cervical secretions (Rutanen et al. 1993, Rutanen et al. 1996). It is suitable for detecting AF in uncertain cases. There is published data on its predictive value as regards preterm rupture of (Rutanen et al. 1993, Rutanen et al. 1996), and a commercial strip test has been developed for its detection. The sensitivity of the test is very high (94–100%), and specificities are around 95% (Rutanen et al. 1996). In clinical applications 74% sensitivity has been documented (Lockwood et al. 1994). In term pregnancies phosphorylated IGFBP-1 seems, on the other hand, to predict inducibility of labor (Nuutila et al. 1999). There is no convincing evidence, so far, that phosphorylated IGFBP-1 determinations in cervical secretions can predict preterm birth, especially before PROM. It has been postulated that by combining different markers, the prediction of preterm birth could be improved (Rizzo et al. 1996).

2.3.2 Cervical status

The role of the cervix and uterus, and their interplay in maintaining pregnancy and in parturition has been much studied (Sonek & Shellhaas 1998). Cervical digital examination, using the Bishop scoring system, evaluates the following parameters: position, consistency, dilatation, effacement of the cervix and the station of the presenting part of the fetus (Bishop 1964). Even this method is, however, to some extent subjective. The adequacy of cervical strength in normal pregnancy has been recognized for centuries; the first description of cervical incompetence appeared in the medical literature in 1658 (Harger 1983). In the early 1960s the hypothesis of premature cervical ripening was addressed, but it was not until 1986 that Bouyer et al. (Bouyer et al. 1986) introduced a scoring system in a study with nearly 8000 women. In this study dilatation of the internal os of the cervix and shortening of the cervix were useful predictors of preterm birth.

Ultrasonographic techniques have come to aid cervical digital evaluation. Transabdominal ultrasonography was initially the evaluating tool for cervix measurement (Sarti et al. 1979). Transperineal ultrasonography has also been used, especially by radiologists (Lewin et al. 1976). Cervical incompetence, seen in the form of bulging membranes, was demonstrated (Vaalamo & Kivikoski 1983). Maternal obesity and bladder filling are, however, pitfalls in this modality. Brown et al. first introduced transvaginal cervical ultrasonography for measuring the cervix (Brown et al. 1986) and since then it has been used as modality in cervical evaluation during pregnancy.

The correlation of ultrasonographic and digital examination has been studied by several authors, with varying results – a fairly poor correlation (Gomez et al. 1994), and also good correlations (Brown et al. 1986). In general it can be said that the cervix seems to be evaluated as being shorter in digital examination than in ultrasonography. Many
investigators have shown that cervical changes referring to incompetence are associated with the risk of preterm birth (Guzman et al. 1994, Timor-Tritsch et al. 1996, Iams et al. 1996). Both the degree of cervical shortening and the magnitude of endocervical funnelling appear to be correlated to the risk of preterm birth. The concept of cervical changes as a continuum rather than an all-or-nothing phenomenon has been shown in a study by Iams et al. (Iams et al. 1996). In this study a clear inverse correlation between cervical length and the risk of preterm birth was found.

At different times of gestation, cervical length is different, and nulliparous and parous women have different cervixes (Cook & Ellwood 1996, Sullivan 1998). Normal cervical length was evaluated in a large trial involving nearly 3700 women between 18 and 22 weeks of gestation, in whom it was mean 4.07 cm, if pregnancy continued to full term (Taipale & Hiilesmaa 1998). In this study a cervical length of 30 mm or less increased the risk of preterm birth over 6-fold, but the positive predictive value was only 13% (the incidence of preterm delivery was only 2.4% in the studied population). The timing of measurement should be 20, 24 and 28 weeks of gestation in cases with a history of previous preterm delivery. Two thirds of preterm births were found in a low-risk population of over 700 women, when a cervical cut-off length of 35 mm was used (Tongsong et al. 1995). In that study, however, funnelling was not measured.

Timor-Trisch et al. found funnelling (Fig. 2) of the cervix to have a 60% predictive value and 100% sensitivity in predicting preterm birth, with a single examination of women admitted to hospital because of cervical change caused by contractions. Transfundal pressure elicits changes in the cervix in the form of increased funnelling (Guzman et al. 1994). It may assist in detecting asymptomatic incompetent cervixes.

Fig. 2. An opening of the cervical internal os- a funnelling.
2.3.3 Fetal surveillance tests

In perinatal medicine fetal risk assessment is of great importance and plays an important role, e.g. in evaluation of indicated preterm birth. Balancing intrauterine and neonatal risks remains an integral part of management decision-making.

2.3.3.1 Conventional fetal monitoring methods

Fetal asphyxia is the major contributing cause of fetal death. Fetal movement monitoring is the simplest method of antepartum fetal surveillance (Pearson & Weaver 1976). At the end of the sixties electronic fetal monitoring was established (Hammacher 1969). Fetal heart rate (FHR) patterns consist of the following components: baseline heart rate, heart rate variability, accelerations and decelerations. Normal neurologic development of the fetus is a prerequisite of short- and long-term variability in normal cardiotocography (CTG). The specificity of a reactive fetal heart rate finding is high, but the positive predictive value is below 40% (Thacker & Berkelman 1986). Computerized CTG analysis was developed during the 1970s and early 1980s to quantify CTG variability, because interpretation by visual assessment is inconsistent (Yeh et al. 1973, Maulik et al. 1983). The computerized system measures pulse intervals that are beat-to-beat times in milliseconds. The Oxford Sonicaid System is based on the work of Dawes and Redman (Dawes et al. 1985). CTG variation declines before variable decelerations occur in growth-retarded fetuses (Snijders et al. 1992). A fetus with an inadequate placental reserve demonstrates late decelerations in response to hypoxia during uterine contractions. There is a low incidence (less than 1%) of antepartum fetal death within one week of a normal finding, but the false-positive rate is reported to be greater than 50% (Garite et al. 1978). Fetal electrocardiography (STAN recorder) analysing ST waveforms combined to CTG analysis seems to identify adverse events during labor (Luzietti et al. 1999).

The biophysical profile score (BPS) measures the fetal response to possible hypoxia. It involves detection of breathing movements, gross body movements, tone, reactive heart rate, and the amount of AF. With this method a dramatic improvement of perinatal outcome (Manning et al. 1980) and reduction of perinatal mortality has been achieved (Baskett et al. 1987). The method has been compared with the non-stress test (FHR acceleration with fetal movement), and found to be more accurate in the diagnosis of fetal asphyxia (Lavery 1982). Gradual hypoxia means that biophysical activities that develop last in utero are the first to become abnormal in the presence of fetal asphyxia (FHR reactivity, breathing movements, body movements, tone, in that order) (Vintzileos et al. 1991).

Amniotic fluid assessment is an important indicator of fetal status. During hypoxemia, fetal renal perfusion decreases, and thence fetal urine secretion decreases, and oligohydramnios develops. A four-quadrant technique (the sum of four vertical quadrants) to constitute the amniotic fluid index was introduced in 1987 (Rutherford et al. 1987). A reduced amount, defined as an amniotic fluid pocket below 1 cm, has a striking positive correlation to adverse perinatal outcome (Chamberlain et al. 1984). By using
only reduced AF as an only modality of antepartum fetal testing, however, no improved neonatal outcomes necessarily result in prolonged pregnancies. On a contrary, a higher rate of CS can be a consequence of increased rate of labor inductions (O'Reilly-Green & Divon 1996). Erythropoetin (EPO) in AF and cord blood is associated in fetal hypoxia, and thence could be used as a marker of asphyxia combined to other fetal monitoring modalities (Teramo et al. 1987, Vora & Gruslin 1998).

Fetal acid-base balance can be assessed antenatally by cordocentesis and during delivery by taking capillary samples, but the former being an invasive method its use is restricted to special circumstances. Intrapartum methods also include fetal pulse oximetry (McNamara & Johnson 1994), the applications of which have met with some technical difficulties.

Intrauterine growth retardation can be a warning of fetal compromise, and it can be detected by ultrasound biometry. In symmetric IUGR, which begins early in gestation and constitutes 30% of all cases of IUGR, the fetus is proportionally small. Underlying causes can be infections or chromosomal disorders, but the fetus can also be normal (Seeds 1984). Asymmetric IUGR (70% of all cases of IUGR) is mostly due to placental insufficiency. Fetal circulatory redistribution under a hypoxic environment is a cause of diminished abdominal circumference. With the aid of ultrasonographic measurements these two conditions can be distinguished. Abdominal circumference correlates well to IUGR (Kurjak et al. 1980).

2.3.3.2 Doppler method

When a transmitted sound wave is reflected from a moving target, the frequency of the transmitted sound wave is altered. This is called a Doppler phenomenon (Gill 1987). The frequency difference is called Doppler shift, which is dependent on the angle of transmitted sound, the direction of a moving target, and on the velocity of the target. In medical use, blood flow measured by Doppler ultrasonography is in the audible range. In blood vessels blood cells are the target and the waveform obtained from arteries represents changes in the velocity of blood during the cardiac cycle.

The blood flow velocity waveform (FVW) is described in terms of pressure and flow. Resistance is defined as the ratio of mean pressure difference across a vascular bed to mean flow through it (Milnor 1972). Resistance depends on arteriolar caliber and distensibility and blood viscosity. The term impedance takes into consideration the pulsatile nature of blood (O’Rourke 1982). The most common indices in obstetric and gynecologic use are the pulsatility index (PI), the resistance index (RI, Pourcelot) and the systolic to diastolic ratio (S/D). They are calculated from the following equations: $PI = (S - D)/mean\ velocity$, and $RI = (S - D)/S$, where $S$ is the maximum Doppler shift and $D$ the minimum Doppler diastolic shift (Thompson et al. 1988).

In the uterine circulation attention has been focused in the evaluation of waveforms on the presence of an early diastolic notch, which has been reported in normal pregnancy upto 20 weeks’ gestation. Its further maintenance is seen in hypertensive disorders of pregnancy and in cases of IUGR (Thompson et al. 1988). It has, however, a high false-positive rate (Zimmermann et al. 1997) in the prediction of pre-eclampsia.
The umbilical cord, linking the fetus and placenta, is ideal for Doppler studies, being suspended in the amniotic fluid, but its spiraling course makes the measurement angle difficult. The umbilical circulation represents a low-resistance vascular bed, through which some 40% of combined fetal ventricular output goes (Rudolph & Heymann 1970). In animal studies it is not until 50% to 60% of the placental vessels are obliterated that the PI is increased. This emphasizes the capacity of the placenta (Thompson & Trudinger 1990). Gestational age (GA) influences its FVW. In early pregnancies diastolic flow may be absent (Nicholaides et al. 1988). Fetal breathing movements alter FVW. During breathing the systolic-to-diastolic ratio is changed (van Eyck et al. 1985).

Abnormalities of the umbilical artery are characterized as patterns of reduced, absent or reversed diastolic flow velocities (Fig. 3). Indices of resistance are thus increased. It has to be stressed that these umbilical indices do not represent the fetal condition but rather placental vascular insufficiency, although this precedes fetal deprivation. Poor fetal growth is the major clinical association (Trudinger et al. 1985, Gudmundsson & Marsal 1988). Among a group of fetuses born SGA, Doppler ultrasonography in the umbilical artery predicted those more likely to require early delivery and time in a neonatal intensive care unit (NICU) and those with the highest mortality (Trudinger et al. 1985).

Careful anatomic ultrasonographic study is necessary because of a 5–10% incidence of anomalies in cases with pathological indices in the umbilical artery (Farrine et al. 1995). Hypertensive pregnancies complicated by IUGR are at an even higher risk of developing AREDV in the umbilical artery, and thus require careful surveillance (Karsdorp et al. 1994). In diabetic pregnancies complications tend to be related to poor glycemic control, and are not predicted by Doppler ultrasonography (Zimmermann et al. 1992). In multiple pregnancies, Doppler monitoring is of value in reducing mortality (Giles et al. 1988). The relationship between Doppler ultrasonography and CTG has been evaluated, and umbilical artery Doppler pathologies appear to select a group of high risk cases. Abnormal CTG findings occur later than abnormal flow in the umbilical artery (Almström et al. 1992).

Fig. 3. A reversed end-diastolic velocity in the umbilical artery.
Fetal arterial studies have been performed mainly in the aortic and cerebral circulations. The midthoracic area of the aorta is free of large branches and measurements at this level are most consistent. The PI in the thoracic aorta does not change with gestation, but is significantly affected by changes in fetal heart rate. It predicts perinatal morbidity and IUGR (Jouppila & Kirkinen 1989). Aortic FVWs seem to provide no additional predictive value over that of the umbilical artery. The peak mean velocity of the aortic waveform, however, correlates with hypoxemia and acidemia determined by cordocentesis (Soothill et al. 1986). In studies on lambs the correlation of blood gas analysis and aortic FVW can be explained by depressed myocardial function and ventricular output of the fetus (Thompson et al. 1994).

The fetal cerebral circulation can easily be observed in the transverse plane, measuring biparietal diameter, when the cavum septum pellucidum and thalamus are included. At the anterior part of the circle Willis is the middle cerebral artery. It demonstrates in normal conditions a relatively high-resistance pattern. In high risk pregnancies the PI becomes lower, demonstrating the brain-sparing effect (van den Wijngaard et al. 1989). When the oxygen deficit becomes worse the PI eventually rises again. Arias et al. (Arias 1994) suggested that at ratio of cerebral to umbilical index of resistance less than 1.0 identifies fetuses at risk. However, the value of cerebral flow findings is limited in assessment of timing of delivery in high-risk pregnancies.

Gembruch et al. (Gembruch & Baschat 1996) recognized a heart-sparing effect, based on observation of increased coronary flow as a manifestation of redistribution of ventricular output in fetuses with severe IUGR. The extremely hypoxic fetus finally has falling contractility and output of the heart. Ultimately, signs of congestive heart failure develop at extreme stress (Räsänen et al. 1997). Central venous pressure reflects cardiac function. Venous Doppler ultrasonographic studies have been the focus of great interest as regards fetal studies in recent years (Rizzo et al. 1996, Kiserud 2000). The ductus venosus, inferior vena cava and umbilical vein have been the main subjects (Hecher et al. 1995). Pulsation in the umbilical vein is associated with fetal compromise (Gudmundsson et al. 1991). It is, however, sometimes seen also in normal pregnancies (Huisman et al. 1994). The explanation for this phenomenon is that there is retrograde flow in the inferior vena cava and umbilical vein during atrial contraction, reflecting impaired capacity of the fetal heart. Umbilical vein flow also seems to be decreased in SGA fetuses (Jouppila & Kirkinen 1983). The trumpet-like vein connecting the portal venous sinus and umbilical vein is called the ductus venosus (Kiserud 1994). In this vessel, the systolic to atrial contraction peak velocity ratio and preload index have been associated with fetal hypoxemia (Kiserud et al. 1991). The ductus venosus to umbilical vein ratio is related to fetal stress (Tchirikov et al. 1998). Flow is always forward in normal ductus venosus; thus retrograde flow during atrial contraction is a sign of fetal compromise.

In clinical decision making, Doppler ultrasonography is of value in detection of chronic fetal distress, but not various acute fetal deteriorations, such as acute hemorrhage. Meta-analysis has established that women with high fetal risk pregnancies should have access to Doppler ultrasonographic studies of the umbilical artery (Alfirevic & Neilson 1995). Such trials have demonstrated a reduction of perinatal death by 38% in these groups. By combining markers of ischemia - such as troponin - to Doppler ultrasonography the prediction of fetal distress could be improved (Mäkikallio 2000).
2.4 Preterm infant

### 2.4.1 Peri- and neonatal mortality

Both perinatal (PNM) and neonatal mortality (NNM) have decreased continuously in Western countries, although the figures are not fully comparable because of statistical differences in reporting, and definitions (Lee et al. 1995, Draper et al. 1999, Koskinen et al. 1999). The largest decline in mortality has occurred in VLBW infants (Hack et al. 1995, Lee et al. 1995, Gissler 1996, Draper et al. 1999). The reason for this improvement is due to overall improved peri- and neonatal treatment, but foremost the use of prenatal corticoids and postnatal surfactant treatment (Crowley 1995, Hamvas et al. 1996). According to the latest Finnish Perinatal Statistics (Koskinen et al. 1999) the PNM rate in Finland was 6.5/1000 births, the NNM rate 2.9 and infant mortality 3.7/1000 live births in 1998. According to birth weight the PNM rate was 102 in the group of those < 2500 g, 369 in the group of those < 1500 g, and 587 in the group of those < 1000 g, per thousand births. The NNM rates were correspondingly 41, 190 and 367/1000 live births. When looking at gestational age, the PNM rate in the group born prematurely (< 37 weeks of gestation) was 76/1000, and it was 333/1000 if delivery occurred at < 32 weeks of gestation. The group of ELBW infants forms one third of the total cases of peri- and neonatal mortality and the group of VLBW accounts for half of the total cases of PNM and NNM, although their proportion of all births is approximately 1%. The PNM rate is higher in multiple pregnancies compared with singletons, and that of boys somewhat higher than that of girls (Koskinen et al. 1999).

The most important causes of death appear to be malformations and asphyxia, and in the group born prematurely, also lung immaturity and RDS (Erkkola & Kero 1991, Koivisto & Pokela 1997). Infection as a cause of death does not appear to be significant in these statistics, but maybe some of the unknown causes could be categorized as being due to infections, which are thought to be etiological factors in a third of preterm births (Lamont & Fisk 1993, Keirse 1995). In a study by Erkkola et al., infections accounted for 12% of deaths in utero of VLBW fetuses (Erkkola & Kero 1991).

### 2.4.2 Neonatal morbidity

Despite the decrease in mortality figures, neonatal morbidity is still high in the lowest weight categories, and it is inversely correlated to birth weight and gestational age at birth (Robertson et al. 1992, Hack et al. 1995, Erkkola & Kero 1991). Typical causes of neonatal morbidity are respiratory problems, hypoxic-ischemic events, intracranial hemorrhage, metabolic disorders such as hypo- and hyperglycemia, hyperbilirubinemia and hypocalcemia, and infections (Robertson et al. 1992, Hack et al. 1995). In the following, respiratory problems, asphyxia, periventricular leukomalacia (PVL), IVH, hypoglycemia and infection are reviewed briefly.
2.4.2.1 Respiratory problems

Respiratory distress syndrome is due to surfactant deficiency and lung immaturity is the most common cause of respiratory insufficiency in the neonatal period (Erkkola & Kero 1991, Robertson et al. 1992, Hack et al. 1995). The greatest risk factor as regards RDS is preterm birth. The incidence varies inversely with gestational age and birth weight (Robertson et al. 1992). Hack et al. (Hack et al. 1995) have reported that 86% of infants of birth weights between 501 g and 750 g, 79% between 751 g and 1000 g, 48% between 1001 g and 1250 g and 27% between 1251 g and 1500 g were noted to have RDS and/or respiratory insufficiency of prematurity. Additional risk factors are male sex, CS, perinatal asphyxia, second twin and maternal diabetes. Other causes of respiratory insufficiency in the preterm infant include transient tachypnea, apnea, pneumothorax and other air leaks, and pulmonary hypoplasia.

Change in obstetric and neonatal care, including the introduction of prenatal corticosteroids and postnatal surfactant and corticosteroids to prevent and treat RDS, have resulted in improved outcomes (Crowley 1995, Banks et al. 1999). In a study by Crowley et al. the incidence of RDS decreased more than 50% if the steroid was administered within an interval of 24 hours to 7 days before birth (Crowley 1995). Postnatal treatment with surfactant decreases the mortality associated with RDS to the same degree (Hamvas et al. 1996). RDS is, however, still the most important cause of death in the neonatal period among infants born preterm (Hamvas et al. 1996, Koivisto & Pokela 1997). One of the most serious complications among the survivors is bronchopulmonary dysplasia (BPD). Others are pulmonary air leaks, infections, and patent ductus arteriosus (Robertson et al. 1992, Hack et al. 1995).

2.4.2.2 Asphyxia, periventricular leukomalacia and intracranial hemorrhage

Threatened fetal asphyxia connected to hypertensive disorders of pregnancy and/or to IUGR, is an important cause of indicated preterm birth. The term asphyxia is imprecisely determined, but its components include hypoxemia, ischemia, hypercarbia and acidosis (Gilstrap et al. 1989). Acute asphyxia can result from perfusion failure because of abruptio placentae or umbilical cord circulation compromise (prolapce or compression). Chronic intrauterine hypoxia can be compensated for by means of cardiovascular and metabolic energy reserves in the fetus. Mild intrauterine stress can enhance lung maturation and be advantageous for the newborn infant (Gross 1990). The fetus can adapt to the chronic situation by redistributing cardiac output to the brain and myocardium (Räsänen et al. 1996). Severe pre- or postnatal asphyxia can cause multiorgan failure and death. If an infant survives, most damage can be repaired, but brain injury can be permanent, leading to hypoxic-ischemic encephalopathy (Volpe 1997). The consequences of hypoxic-ischemic insults in preterm and full-term infants are somewhat different because of the degree of brain maturation (Volpe 1995). In the former the most vulnerable area is cerebral white matter, whereas in the latter, it is the cerebral cortex.
Focal or diffuse PVL is the most usual cerebral white matter injury seen in preterm infants after cardiorespiratory insufficiency. Its incidence varies greatly according to gestational age. In a large series of infants born at gestational age below 33 weeks, the overall incidence was 9.2%, with the lowest incidence of 4.2% at 32 weeks' gestation (Volpe 1997). Current concepts of the pathogenesis of PVL are related to three major interacting factors. Firstly, maturationally underdeveloped arterial end and border zones in deep periventricular white matter, i.e., watershed areas, are vulnerable if cerebral blood flow decreases. Secondly, cerebral vascular autoregulation is impaired in certain preterm infants and consequently, they have a pressure-passive cerebral circulation. The third important factor of PVL is related to an intrinsic vulnerability of immature cerebral white matter to different harmful agents, e.g. free radicals and cytokines (Volpe 1997, Volpe 1998). The incidence of PVL is higher in cases of maternal chorioamnionitis (Perlman et al. 1996, Yoon et al. 1996). A long-term consequence of PVL is typically spastic diplegia.

In addition to PVL, intracranial hemorrhage in the perinatal period play a very important role in the development of cerebral palsy in preterm infants. Germinal matrix intraventricular hemorrhage (IVH) is the most common type of intracranial hemorrhage in these infants. The incidence of IVH is inversely correlated to gestational age and birth weight (Robertson et al. 1992, Hack et al. 1995). The germinal matrix is a vasculature-rich tissue, which diminishes towards term. Bleeding originating from the subependymal germinal matrix extends to the ventricular or parenchymal regions. According to the current concept approximately 15% of infants with IVH also develop periventricular hemorrhagic infarction, possibly because of venous obstruction, which in turn often has adverse consequences on motor and intellectual development (Volpe 1997, Volpe 1998). IVH occurs in the first postnatal 24 hours in 50% of the cases and by the age of 3 days 90% of cases of IVH can be detected (Roland & Hill 1997).

Corticosteroids stabilize cell membranes, and numerous studies indicate that antenatal corticosteroids reduce mortality as well as the incidence and severity of IVH (NIH 1995). Indomethacin used in tocolytic therapy has been claimed to increase the risk of IVH (Norton et al. 1993), but also opposite findings exist (Gardner et al. 1996, Vermillion & Newman 1999). The role of cesarean delivery in reducing the risk of IVH was controversial until there was evidence from studies (Levinton et al. 1991, Ment et al. 1992, Shaver et al. 1992) showing that low birth weight infants are at risk of IVH in prolonged vaginal delivery. Postnatal correction of hemodynamic disturbances and adequate ventilation prevent the risk of IVH.

### 2.4.2.3 Hypoglycemia

Hypoglycemia is seen more often in SGA infants and prematurely born infants than normal weight and term infants (Lubchenco & Bard 1971, Robertson et al. 1992), because of reduced placental supplies (Koivisto & Jouppila 1974). It is seen in infants of diabetic mothers, whose hyperglycemia leads to fetal hyperinsulinemia and which may precipitate a decrease in postnatal glucose. β-Adrenergic agents used as tocolytics can cause maternal hyperglycemia, and preterm infants exposed to this treatment may suffer hypoglycemic consequences. Other causes of hypoglycemia due to altered glucose
production in preterm infants are lack of glycogen storage in SGA infants, as well as decreased gluconeogenesis from amino acids. These infants often have increased peripheral glucose use because of hypoxia and infection (Williams et al. 1975).

Definitions of hypoglycemia vary between 1.7–2.5 mmol/L during the first three days of life.

Koh et al. found changes in brain stem auditory-evoked responses in infants who had blood glucose levels below 2.5 mol/L (Koh et al. 1988). There is a distinct correlation between neonatal symptoms and signs, and later outcome. The outcome is worst if hypoglycemia causes seizures (Koivisto et al. 1972), but even moderate hypoglycemia seems to expose infants to adverse neurodevelopmental outcome (Lucas et al. 1988).

2.4.2.4 Neonatal infection

Newborn infants and especially preterm infants, are prone to infections, because their own and their maternally acquired immunity is inadequate. Infections are also often present in cases of preterm labor. Etiological bacteria consist mainly of group B streptococci, which accounts for 1–2 cases of fetal/neonatal sepsis per 1000 births (Boyer 1995). Early onset (< 7 days) GBS presentation is pneumonia and septicemia, and late onset (> 7 days) infection often occurs as meningitis. In early onset sepsis, GBS has been found to be major pathogen (29%), followed by Staphylococcus aureus (15%) and Escherichia Coli (14%) (Vesikari et al. 1989). Infections are more frequent and more often lead to death in preterm infants than in term infants (Erkkola & Kero 1991, Robertson et al. 1992).

2.4.3 Long-term outcome

2.4.3.1 Chronic lung disease

Some neonates develop a form of chronic respiratory disease. Northway first described bronchopulmonary dysplasia (BPD) in preterm infants following prolonged mechanical ventilation with high airway pressure and high inspired oxygen concentration (Northway et al. 1967). This initial work described the course of the disease through four stages beginning at the age of two to three days, and reaching the final chronic phase beyond the age of one month. Because chronic lung injury can develop in preterm infants without severe or any RDS, the common term chronic lung disease (CLD) is used and its definition is based on clinical criteria only, at either 28 days of age or 36 weeks of adjusted gestational age.

The incidence is variable because of differences in definition, but the highest incidences are reported in the lowest birth weight groups (Avery et al. 1987, Rojas et al. 1995). In a recent study, a need for supplemental oxygen was seen at the age of 28 days in
31% of surviving infants with a birth weight below 1501 g, but at the age of three months only in 13% of these infants (Korhonen et al. 1999).

The cause is multifactorial. In addition to prematurity, high oxygen concentration, high ventilator pressures, endotracheal intubation, infection, patent ductus arteriosus and excessive fluid administration increase the risk of CLD (Tammela & Koivisto 1992, Barrington & Finer 1998).

Chronic lung disease is a marked problem in preterm infants, because of an association with chronic respiratory difficulties, prolonged and recurrent hospitalization, an increased incidence of neurodevelopmental disabilities, growth failure and, at its severest, death (Barrington & Finer 1998). The long-term pulmonary outcome of prematurely born infants with BPD has been investigated in some studies. Northway (Northway et al. 1990) performed such a follow-up study at the age of twenty years, and found that a quarter still had respiratory symptoms. Bronchial hyper-reactivity, lability and bronchial obstruction seem to be very common at school age in children born preterm and surviving from BPD (Pelkonen et al. 1997).

2.4.3.2 Neurological outcome

The incidence of cerebral palsy (CP) and minor neurological disorders of preterm infants is inversely related to birth weight and gestational age (Hack et al. 1994, Tin et al. 1997). Incidences of CP in VLBW children vary in different countries between 5–19% (Ikonen et al. 1992, Hagberg et al. 1996). Recently, Salokorpi et al. (Salokorpi et al. 1999) found a 17% incidence of CP in preterm children with a birth weight of 1000 g or less in Finland. There has been some controversy concerning whether or not the incidence of neurological morbidity has increased with decreasing perinatal mortality. It seems that in the long term, the improved survival of VLBW infants has not been accompanied by an increase but rather by a decrease in the incidence of neurological disorders (Järvenpää et al. 1991, Lee et al. 1995), although in some studies contrasting results have been found (Escobar et al. 1991, Hagberg et al. 1996). Examinations of prematurely born children at school-age have revealed that they have disturbances, especially in cognitive ability and visuomotor integration, more often than full-term counterparts (Hack et al. 1994, Olsen et al. 1995, Korkman et al. 1996).

2.4.3.3 Growth

Preterm delivery is characterized as an independent risk factor associated with suboptimal fetal growth in many studies (Tamura et al. 1984, Weiner et al. 1985, Ott 1993). Pathology in trophoblast invasion in pre-eclampsia is an underlying cause of placental insufficiency, and the diminished blood flow in redistributed fetal circulation by compensatory brain-sparing mechanisms may lead to IUGR. In PPROM cases, oligohydramnios by means of restricting space, brings about hypoplastic changes in the fetus (Nimrod et al. 1984), or asymmetric growth failure in late pregnancy (Hediger et al. 1992).
1995). Other factors affecting growth include infections and drugs (e.g. smoking, alcohol). Later, however, these infants show catch-up growth. This refers to rates of growth over a certain time period that are greater than the usual rate of growth (Elliman et al. 1992).

Hack et al. found catch-up growth in all parameters at 8 years of age among VLBW infants (Hack et al. 1996). Full-term SGA infants are obese at five years after catch-up growth by two years (Ong et al. 2000). In another study adolescent cases of ELBW, who were born SGA, attain lower growth measurements compared with normal birth weight adolescents (Peralta-Carcelen et al. 2000).

Recently, interest has arisen in IUGR and its ramifications to later adult disease (Forsen 2000). Barker (Barker et al. 1993), in a British population, showed an increase in coronary heart disease among low birth weight men. The mechanisms behind this are unknown. Maybe deficient nutrition affects organ function or resets the levels of hormones affecting growth. Impaired fetal heart function in asphyxia may lead to failures in later life.
The aims of the present study were:

1. To evaluate the prevalence of BV in early pregnancy, and the effect of intervention with vaginal clindamycin in low-risk pregnant women on the preterm birth rate.
2. To investigate the predictive value of the cervical biochemical markers IL-6, IL-8 and IGFBP-1, and cervical ultrasonography, and their combination in risk assessment of preterm birth.
3. To investigate the clinical significance of early (before 34 weeks of gestation) detected AREDV in the umbilical artery on perinatal outcome.
4. To evaluate neonatal outcome, and late sequelae at one year of corrected age in infants delivered after indicated preterm birth due to maternal or fetal reasons.
5. To evaluate peri- and neonatal outcome, and late – especially pulmonary – of infants born preterm due to PPROM, at one year of corrected age.
4 Subjects and methods

The present study involves five groups of subjects. It was performed at the Departments of Obstetrics and Gynecology, and Pediatrics, Oulu University Hospital, during the years 1995–2000. The Ethics Committees of the Medical Faculty of Oulu University and the Health Center of Oulu city approved the study protocol. For the prospective part of the study, informed consent from the subjects was obtained.

4.1 Subjects

Subject characteristics are summarized in Table 1.

Study I: 1956 singleton pregnancies without previous preterm delivery, in 17 health centers of Oulu from March 1996 until March 1998, were screened at 12 weeks of gestation for BV at the first antenatal visit. One hundred and one were randomized at Oulu University Hospital to receive vaginal clindamycin, or placebo. Control swabs were taken one week after treatment and again at 30 gestational weeks. The main outcome investigated was preterm birth.

Study II: from February 1997 to July 1998, 77 women with singleton pregnancies at 22–32 weeks of gestation, with premature contractions and/or cervical change, and 78 controls matched for age (under 20 years, from 20 to 35 years, and over 35 years of age), parity (primiparous, parity from 1 to 4, and parity over 4) and gestational weeks (+/-one week), were prospectively followed-up three times at two-week intervals. Cervical samples for determinations of IL-6, IL-8 and IGFBP-1 were taken and transvaginal cervical ultrasonography was performed at these visits. The predictive value as regards of preterm birth of these single tests and by combining them was analyzed.

Study III: Eighty-three women with hypertensive disorders of pregnancy or an SGA fetuses with AREDV in the umbilical artery detected by ultrasonography before 34 weeks of gestation were retrospectively analyzed 1988 to 1995 as regards perinatal outcome. Multiple pregnancies were excluded.

Study IV: One hundred and three women between the 24th and 33rd week of pregnancy, delivered by CS because of maternal or fetal indications and 103 matched controls (gestational age +/- one week) with spontaneous preterm delivery in 1990–1997 were
retrospectively analyzed. Singleton pregnancies, where the fetus was alive at the time of the decision to carry out cesarean delivery, were included in the study group. Maternal morbidity, peri- and neonatal outcomes as well as the pulmonary and neurological sequelae and growth of the infants at the age of one year were analyzed.

Study V: included a retrospective cohort of 78 women with singleton pregnancies who delivered between 1990 and 1996, and who had PPROM between the 17th and 30th gestational weeks matched with 78 controls with spontaneous preterm delivery during the same period. Women delivering within 24 hours after PPROM were excluded. Peri- and neonatal mortality and morbidity and especially the pulmonary outcome of the infants were analyzed up to one year of corrected age.

Table 1. Summary of the study populations in studies I-V (values given are as numbers of women (percent), age in mean and standard deviation (SD)).

<table>
<thead>
<tr>
<th>Study group</th>
<th>Number</th>
<th>Maternal age (y)</th>
<th>Previous preterm delivery (%)</th>
<th>Maternal hypertensive disorders (%)</th>
<th>Bleeding at 1st trimester (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td>-screened</td>
<td>1956</td>
<td>27.9 (5.2) 27.8 (5.8)</td>
<td>2 (1.6) 0 (0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-randomized</td>
<td>62</td>
<td>29.9 (6.3) 30.8 (6.0)</td>
<td>19 (25) 3 (4) *</td>
<td>6 (8) 2 (3) 22 (29) 20 (26)</td>
</tr>
<tr>
<td>Study II</td>
<td>77</td>
<td>27.9 (6.3) 30.8 (6.0)</td>
<td>19 (25) 3 (4) *</td>
<td>6 (8) 2 (3) 22 (29) 20 (26)</td>
<td></td>
</tr>
<tr>
<td>Study III</td>
<td>83</td>
<td></td>
<td></td>
<td>64 (77)</td>
<td></td>
</tr>
<tr>
<td>Study IV</td>
<td>103</td>
<td>32.1 (6.6) 27.9 (6.0)*</td>
<td>25 (24) 24 (23)</td>
<td>59 (57) 1 (1)* 8 (8) 17 (17)</td>
<td></td>
</tr>
<tr>
<td>Study V</td>
<td>78</td>
<td>30.1 (5.3) 28.8 (6.1)</td>
<td>10 (13) 14 (18)</td>
<td>21 (27) 11 (14)</td>
<td></td>
</tr>
</tbody>
</table>

*statistically significant difference between patients and controls

4.2 Methods

Main methods and outcome measures are shown in table 2.

Study I: Samples for Gram staining were collected with a cotton swab from the posterior vaginal fornix, smeared on objective glass, and air-dried. Chlamydial samples were taken with urethral and cervical cotton swabs, and streptococcal samples with vaginal swabs. Interpretation of stains was carried out by using Spiegel’s criteria (Spiegel et al. 1983). Non-spore-forming Gram-positive rods of the lactobacillus type were considered as normal vaginal flora, whereas stain-negative or variable rods of the Gardnerella type were indicative of BV. Gram-intermediate findings consisted of different morphotypes, not specific for BV. A reference strain was used when interpreting stainings. Chlamydia trachomatis was diagnosed by a polymerase chain reaction-based method (Cobas Amplicor, F. Hoffman-LaRoche, Basel, Switzerland). N. gonorrhoeae and GBS were cultured on selective media.
Randomization was accomplished by using blocks created by a pseudo-random number generator. Serially numbered containers were dispensed by the pharmacists without the clinicians’ involvement. Double-blind codes were kept sealed in opaque envelopes.

Clindamycin or apparently identical placebo cream was applied intravaginally for one week, 5 g a day.

Study II: Cardiotocography for registration of uterine contractions for 30 minutes was carried out. A urine sample for bacterial culture was collected. Cervical samples for assays of IL-6 and IL-8, and the decidual isoform of IGFBP-1 were taken with a dacron swab. This was kept in the cervix for 10 seconds, and thereafter placed in 0.5 ml buffer solution, rinsed and removed. The sample was stored at -20 °C. A Pap smear was taken with a wooden spatula from the vaginal fornices and ectocervix, and an intracervical sample with a brush, and fixed with ethanol. Pap smears were analyzed by a single investigator. Chlamydial and streptococcal samples were analyzed as in study I. The concentrations of interleukins-6 and -8 (Immulite System, Diagnostic Products Corporation, LA, USA) and phosphorylated isoforms of IGFBP-1 (Immunoenzymometric assay, Medix Biochemica, Kauniainen, Finland) were determined by commercial assays. Transvaginal ultrasonography was performed with Toshiba SSA-270 equipment, fitting the 5–7 MHz rubber-covered and gel-lubricated vaginal probe into the anterior fornix of the vagina. Cervical length, and possible funneling (widening of the internal os) were measured first at rest. Cervical index was determined (Fig. 4). Next, transfundal pressure was maintained and the same measures repeated.

Table 2. Summary of the main methods and outcome measures used in studies I-V.

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Main outcome</th>
<th>Intra/interassay coefficient of variation (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Gram stain of vaginal swab random allocation to treatment</td>
<td>preterm birth (&lt;37 weeks) puerperal infections</td>
<td></td>
<td>Spiegel et al. 1983</td>
</tr>
<tr>
<td>II</td>
<td>IL-6, IL-8 (chemiluminescence assay) IGFBP-1 (IEA) vaginal ultrasonography</td>
<td>preterm birth puerperal infections neonatal infections</td>
<td>6.0/10.0 4.6/6.4</td>
<td>Nuutila et al. 1999</td>
</tr>
<tr>
<td>III</td>
<td>retrospective data collection on cases with AREDV in the umbilical artery seen before 34 weeks of gestation</td>
<td>perinatal outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>retrospective data collection on cases with indicated delivery before the 33rd weeks of gestation</td>
<td>maternal morbidity neonatal and long-term outcome of infants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>retrospective data collection on cases with PPROM before the 30th weeks of pregnancy</td>
<td>peri- and neonatal outcome pulmonary outcome of infants</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IEA = immunoenzymometric assay, ARED = absent or reversed end-diastolic velocity, PPROM = preterm premature rupture of membranes.
Study III: Pregnancy history and perinatal outcome of the infants were retrospectively collected from hospital records. The Doppler examinations were done with Toshiba SSA-270 ultrasonographic equipment fitted with a 3.5 MHz transabdominal convex probe. Pulsed ultrasound was used with a 120 kHz filter. Umbilical artery measurements were performed several times from the free loop of the vessel at different points. The systolic to diastolic ratio (S/D) was measured, and the possible presence of AREDV was based on visual evaluation. AEDV is a condition in which peripheral resistance in the placenta is increased, so that forward flow through the umbilical artery to the placenta is not achieved throughout the entire cardiac circle. Further increase in resistance results in reversal of flow (REDV) in diastole, such that flow is turned towards the fetal heart. SGA fetuses were defined if the birth weight of the fetus was 2 standard deviations or more below normal (Pihkala et al. 1989).

Study IV: Data on maternal morbidity and treatment during pregnancy and the main reasons for CS were collected from hospital records, as were mortality and neonatal morbidity and late sequelae of infants up to one year of corrected age. In CTG a constant decrease in fetal heart rate variability was defined as low short-term variation (< 4 ms). The appearance of late (after uterine contractions) or variable decelerations (> 20 lost beats) was also considered pathological. In some cases, computer-based analysis of CTG (Team 8002 or Team care, Sonicaid, Oxford) was carried out. Oligohydramnios was defined if the vertical length of the highest fluid column was < 2 cm (Vintzileos & Knuppel 1995).

Diagnosis of RDS was made if there was a need for respiratory support, if there were typical radiologic findings and by clinical assessment. Pulmonary air leak consisted of pneumothorax and pulmonary interstitial emphysema. BPD was diagnosed according to the radiologic criteria proposed by Northway (Northway et al. 1967). Chronic lung disease was defined if the infants required oxygen, and continuous bronchodilator or steroid treatment because of respiratory signs and symptoms at one year of corrected age. Pulmonary hypoplasia was based on autopsy in dead patients, and on compression deformities and unresponsiveness to surfactant treatment in living infants. Central
nervous system abnormalities were detected in cranial ultrasonography or at autopsy. IVH was graded into four categories according to Papile (Papile et al. 1978). PVL was defined, if periventricular white matter cysts were seen in ultrasonography.

Infants were grouped into four categories according to their neurologic outcome: i) normal, ii) delayed motor development with abnormalities of tone or reflexes but functionally normal or borderline, iii) cerebral palsy with spastic diplegia, hemiplegia or tetraplegia and iv) other impairment (e.g. Down’s syndrome).

Measurements of weight, length and occipitofrontal circumference were made by using standardized instruments. Length and head circumference were expressed as standard deviations of normal growth, and weight was expressed relative to length (Piekkala 1994).

Neonatal hypoglycemia was defined if a blood glucose concentration of < 2.5 mmol/L (45 mg/100 mL) more than once was detected and its duration was registered as the complete period until the day the last low blood glucose value was recorded. Neonatal infection was based on positive blood culture, clinical manifestation and laboratory testing (CRP over 20 mg/L) (Da Silva et al. 1995) and in dead patients on histopathological findings.

Study V: Clinical data on the mothers and infants were collected as in study IV. Rupture of the membranes was defined clinically if amniotic fluid was seen pooled in the vagina and diminished AF was seen in ultrasonography, or in uncertain cases by use of the PROM test (Rutanen et al. 1993) (IGFBP-1-based, Medix Biochemica, Kauniainen, Finland) or the nitrazine test. C-reactive protein (CRP) tests, leukocyte counts and axillary temperature recordings were performed daily. Samples for chlamydia, gonococci, streptococci, Pap smear and urine bacteria were obtained at admission. CTG was carried out daily, or in early weeks fetal heart rate was registered by using Doppler ultrasound equipment. Serial ultrasonography was performed at least once a week, and a rough estimate of the amount of AF was made, as well as fetal structure, size and BPS.

Chorioamnionitis was diagnosed if at least two of the following criteria were fulfilled: body temperature higher than 38 °C, CRP level over 20 mg/L, maternal or fetal tachycardia, uterine tenderness and foul smelling amniotic fluid. No prophylactic antibiotics were used and tocolytics and dexamethasone were administered over 2 days on an individual basis. Puerperal infections were defined as an episiotomy or a CS wound infection or endometritis necessitating antibiotic treatment.

4.3 Statistical methods

Statistical analysis was performed with SPSS for Windows (version 7.0-9.0) in all studies except study III, in which Statview for Macintosh was used. The CIA program (Confidence Interval Analysis by Altman D, Gardner, BMJ, 1989) was used. STATA statistical software release 5.0 (Stata Corp, College Station, TX) was used in addition in study V.

Student’s t-test was used for normally distributed continuous variables. In study II logarithmic transformation was used on skewed data before using the t-test. In studies III & IV non-normally distributed data were tested with the Mann-Whitney U-test. In study
V pair continuous variables were tested with the paired t-test. Categorial data were tested with Fisher's two-tailed exact chi-squared test in studies III–V, in addition to which paired categorial data in study V were tested with a sign test.

Receiver-operator characteristic (ROC) curve analysis was carried out in study II in order to determine the cut-off levels reflecting the best sensitivity and specificity of the parameters studied. Sensitivity reflects the true positive rate (the percentage of patients with disease who have a positive test result): true positives/(true positives + false negatives), whereas specificity is the true negative rate (the percentage of patients without disease who have a negative test result): true negatives/(true negatives + false positives). Positive predictive value (PPV) is calculated: true positives/(true positives + false positives) and negative predictive value (NPV): true negatives/(true negative + false negatives). On the ROC curve sensitivity vs. 1-specificity is plotted, the y-axis being the true-positive fraction and x-axis the true-negative fraction. Stepwise logistic regression was used in studies II and IV to see the effects of combinations of independent explanatory factors on the outcome measure. An analysis of repeated measures was also performed (p-values) in study II. Odds ratios (ORs) and 95% confidence intervals (CIs) are reported in the regression analysis. In study I the CIA program was used, and in study V 95% CIs for differences in means were calculated. Relative risks (RRs) were calculated by CIA in study IV. P < 0.05 was used as the level of significance and 95% CIs not including one (in ORs and RRs) or zero (in differences) were considered statistically significant.

The intention-to-treat principle was used in study I, taking care that every subject randomized was followed up and analyzed. The sample size was estimated to be 600 according to the hypothesis that treatment could reduce the incidence of preterm birth by 30%. The rate of preterm birth was estimated to be 30%, based on the findings of a six-fold increase in preterm births in BV patients (Kurki et al. 1992). During the study period there should have been 640 BV positives in the Oulu area, if the prevalence of BV is around 20%.
5 Results

5.1 Early pregnancy bacterial vaginosis and the effect of vaginal clindamycin on preterm birth rate and puerperal infections (I)

The prevalence of BV was 7.3% (143/1956). One-hundred-and-one of the BV positives were randomized to receive either vaginal clindamycin or placebo, and 22 of the BV intermediates (Fig. 5). The preterm birth rate in the BV positives was 9.9% (10/101), and together with the BV intermediates it was 9.8% (12/123). Preterm birth rate in the clindamycin group was 14% (7/51), vs. 6% (3/50) in the placebo-treated subjects (OR 2.5, 95% CI 0.6–10).

![Trial profile in BV study.](image)

* bacterial vaginosis positive, # bacterial vaginosis intermediate.

Fig. 5. Trial profile in BV study.
If BV was detected at least once after randomization, compared with not persisting at all, there were 21% (6/29) preterm births vs. 0% (0/26), respectively (p < 0.01). BV was absent just after treatment in 33% (7/51) of the clindamycin-treated patients vs. 34% (17/50) of placebo-treated patients (OR 1.0, 95% CI 0.4–2.2).

Altogether, 14/123 (11%) cases of puerperal infections were seen. There was a difference in puerperal infectious morbidity in patients where BV persisted (31%, 9/29) compared with those in which BV did not persist (8%, 1/26) (OR 5.4, 95% CI 1.04–28). Infections were seen in 8% (4/51) of the clindamycin-treated vs. 20% (10/50) of the placebo-treated cases (OR 0.3, 95% CI 0.1–1.2).

The above-mentioned figures were similar when the randomized BV+ and BV intermediate cases were analyzed together or separately, or if missing or lost response was included.

5.2 Prediction of preterm birth with cervical IL-6, IL-8, IGFBP-1 and transvaginal cervical ultrasonography, and their combinations (II)

The preterm birth rate among study the subjects was 16% (12/77). One preterm delivery was due to induction of delivery because of pregnancy induced cholestasis. Two preterm infants were also born in the control group. Among the lower social class subjects the preterm birth rate was 18% (10/57) compared with 0% (0/15) in upper level employees. In the group with a history of previous preterm delivery the preterm birth rate was 32% (6/19) vs. 9% (5/58) in the non-previous preterm group (OR 4.9, 95% CI 1.3–19). In women with first trimester bleeding there were 6/22 (27%) preterm births compared with 9% (5/54) without bleeding (OR 3.7, 95% CI 0.99–14). Age over 35 years increased the frequency of preterm births; 26% (6/21) compared with 11% (6/56) of mothers younger than that (OR 4.1, 95% CI 1.1–15).

Cervical IL-6, according to ROC curve analysis, had the best cut-off at 128 ng/L at the second follow-up. As a univariate parameter this test had a sensitivity of 73% (8/11) and a specificity of 77% (50/65) in prediction of preterm birth, which occurred in 35% (8/23) vs. 6% (3/53) of cases, according to whether or not the level was exceeded (Table 3). IL-8, at a cut-off level of 4250 ng/L (second follow-up), had a sensitivity of 73% (8/11) and a specificity of 65% (42/65). IGFBP-1 had the lowest sensitivity (45%; 5/11) but the best specificity (84%; 54/64) of these markers when used as a univariate parameter at a cut-off level of 6.6 µg/L (last follow-up). Preterm birth occurred in 33% (5/15) vs. 10% (6/60) in women with a cervical IGFBP-1 level above this value.

Ultrasonographic measurements were also found to have significant predictive value. Cervical length had its best cut-off value (29 mm) at the second follow-up (with transfundal pressure), with a sensitivity of 52% (34/65) and a specificity of 82% (9/11). Preterm birth occurred in 23% (9/40) vs. 6% (2/36), if the cervix was shorter than that. Funneling at the second follow-up (with transfundal pressure) had a sensitivity of 64% (7/11) and a specificity of 70% (45/64). If a funnel was seen there were 7/26 (27%) vs. 4/49 (8%) preterm births (OR 4.1, 95% CI 1.1–16). A cervical index > 0.2 (at second follow-
up at rest) had a sensitivity of 64% (7/11) and specificity of 77% (50/65). Thirty-two percent (7/22) preterm births vs. 7% (4/54) were seen, according to whether or not this index value was exceeded.

Logistic regression analysis revealed that IL-6 at a cut-off level of 128 ng/L (OR 12.1, 95% CI 2.2–67) combined with cervical index > 0.2 (OR 7.9, 95% CI 1.5–42) were the strongest determinants for preterm birth. This combination had a specificity of 97% and a sensitivity of 46%. When IGFBP-1 (cut-off 6.6 µg/L) was combined with cervical index > 0.2, the specificity of the test “battery” was as much as 98% (OR 6.3, 95% CI 1.3–30), but the sensitivity fell to only 27%.

Puerperal infections were clearly associated with elevated IGFBP-1 concentrations. At a cut-off level of 21µg/L at the first follow-up, 36% (4/11) vs. 3% (2/65) of cases of infection in the puerperium were predicted (OR 18, 95% CI 2.8–117). The same association was seen in neonatal infections, which were seen in 36% (4/11) of the cases if IGFBP-1 (cut-off 21.6 µg/L) was elevated vs. 2% (1/66) if the level was lower than that (OR 37, 95% CI 3.6–380). The controls showed a similar trend as regards infectious morbidity; neonatal infections among controls were seen in 45% (3/62) of the infants if the IGFBP-1 level exceeded 5.2 µg/L, vs. 13% (2/15) if not (OR 3.0, 95% CI 0.5–20).

Table 3. Risk factors for preterm birth (n = 11) among subjects (n = 77) in study II.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical IL-6 &gt; 128 ng/L</td>
<td>73%</td>
<td>77%</td>
<td>8.9</td>
<td>2.1–38</td>
</tr>
<tr>
<td>Cervical IL-8 &gt; 4250 ng/L</td>
<td>73%</td>
<td>65%</td>
<td>4.9</td>
<td>1.2–20</td>
</tr>
<tr>
<td>Cervical IGFBP-1 &gt; 6.6 µg/L</td>
<td>45%</td>
<td>84%</td>
<td>4.5</td>
<td>1.2–18</td>
</tr>
<tr>
<td>Cervical length &lt; 29 mm</td>
<td>52%</td>
<td>82%</td>
<td>4.9</td>
<td>0.99–25</td>
</tr>
<tr>
<td>Funneling</td>
<td>64%</td>
<td>70%</td>
<td>4.1</td>
<td>1.1–16</td>
</tr>
<tr>
<td>Cervical index &gt; 0.2</td>
<td>64%</td>
<td>77%</td>
<td>5.8</td>
<td>1.5–23</td>
</tr>
<tr>
<td><strong>Multivariate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined cervical index &gt; 0.2</td>
<td>46%</td>
<td>97%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and IL-6 &gt; 128 ng/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined cervical index &gt; 0.2</td>
<td>27%</td>
<td>98%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>and IGFBP-1 6.6 µg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.3 The clinical significance of AREDV in the umbilical artery in high risk pregnancies before the 34th week of gestation (III)

Hypertensive disorders complicated the pregnancy in 77% of cases (64/83). The median interval from detection of AREDV to delivery was seven days (range 1–52). The median interval between the detection of AREDV and the first pathological CTG finding was 2 days (range 1–37). Altogether, 70 (84%) CSs were performed. The main indication for delivery was fetal in 58 of the cases and maternal in 15 cases. One-minute Apgar scores below 7 occurred in 77% (10/13) of the REDV group vs. 40% (21/53) of the AEDV group (p = 0.04). There was an 82% (78 cases) rate of IUGR infants.
The PNM rate in the whole group was 19% (16/83), with six stillborn and ten neonatal deaths. One infant died at three months of age. The main cause of death was anomaly (40%, 8/20), and the second major cause was RDS (35%, 7/20). The PNM rate was 11% (7/64) in the group of hypertensive mothers, compared with 53% (10/19) in the rest of the group (p = 0.003). No difference between PNM rates was noted when comparing fetal and maternal indications as the reason for delivery; nor did they differ between AEDV and REDV groups, but birth weight was significantly smaller in the REDV infants (median 1000 g) compared with those with AEDV (median 1360 g; p = 0.02). When anomalies were excluded, the PNM rate was 9% in the AEDV group compared with 36% in the REDV group (p = 0.03). When the interval from AREDV detection to delivery was within three days (n = 44) compared with those in whom this period was longer (n = 39), there were no differences in the PNM rate, although the Apgar scores were lower in the first-mentioned group.

5.4 Indicated preterm birth for maternal or fetal reasons (IV)

The mothers in the indicated group were older (mean 32 versus 28 years) and had more chronic illnesses (mainly hypertensive disorders) compared with the spontaneous group (32% vs. 13%, RR 2.5, 95% CI 1.4–4.5). Gastric carcinoma was the cause of death of one mother who died a month after delivery in the indicated delivery group.

Fetal asphyxia was present according to Doppler ultrasonographic and CTG findings in 50% of the cases in the indicated group. Nearly two thirds of the Doppler pathology group fetuses also had warning signs in CTG. Cesarean section was performed on all in the indicated group, and on 28% (29/103) of the control group. Primary indication for CS was fetal compromise in both groups (61% in the indicated group and 97% in the control group).

Infants in the indicated group were significantly smaller than those in the control group (58% vs. 7% of SGA cases, RR 8.6, 95% CI 4.1–18) and more asphyxiated according to umbilical artery pH (mean 7.23 vs. 7.29, p = 0.002). The NNM rate was higher in the indicated group (175 vs. 78 per thousand live births, RR 2.3, 95% CI 1.02-4.9). RDS occurred more often (73% vs 53%, RR 1.4, 95% CI 1.1–1.7) and it was more severe and more complicated in infants in the indicated group compared with those in the control group (Table 4). RDS was a major death cause and it was seen more frequently if CS was carried out because of maternal (88%) compared with fetal (65%) indications (p = 0.02). Hypoglycemia was more common in indicated preterm infants than in the controls.

At one year CLD was seen in 15% of the indicated group infants compared with 3% in the controls (RR 2.7, 95% CI 1.4–16). The incidence of CLD (22%) was higher in the AREDV group (8/37), compared with 5% in the non-AREDV group (6/112; RR 2.7, 95% CI 1.5–4.6). The indicated group infants were still smaller than their spontaneous counterparts but the neurologic outcome did not differ (Table 5).
5.5 Perinatal and neonatal outcome and late sequelae of infants born after PPROM (V)

PPROM mothers had a higher frequency of chronic diseases compared with the controls (20% vs. 12%, OR 1.9, 95% CI 0.8–5.1). Previous cervical operations and current pregnancy bleeding in the first trimester were more common among PPROM mothers (6% vs. 0% and 25% vs. 14%, respectively). Histopathological chorioamnionitis was seen in 51% of the PPROM group vs. 29% of the control group (OR 2.5, 95% CI 1.3–4.9). PPROM occurred at a median of 25.9 weeks (range 17.8–29.8) and the median interval from PPROM to delivery was 7 days (range 1–92).

The PNM and NNM rates did not differ between the groups. Respiratory problems and infections were the commonest causes of death in both groups. Infants born after PPROM were smaller than their control counterparts. There were more limb contractures in PPROM infants compared with the controls (6 vs. 0 cases) and pulmonary hypoplasia was also detected more frequently in PPROM infants (9 vs. 0 cases). Otherwise, there were no differences in neonatal morbidity between the groups (Table 4).

Long-term follow-up until one year of corrected age revealed that the PPROM infants needed more rehospitalization days because of lung problems than the controls (5 vs. 1 days) and CLD was more common in these infants (22% vs. 9%, OR 2.4, 95% CI 0.9–6.5). No differences were detected in neurosensory development. At one year of corrected age PPROM infants tended to be smaller than their control counterparts (Table 5).

Table 4. Morbidity of liveborn infants during primary hospitalization in studies IV and V.

<table>
<thead>
<tr>
<th>Neonatal outcome</th>
<th>Study IV</th>
<th></th>
<th>Study V</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients (n = 102) n (%)</td>
<td>Controls (n = 103) n (%)</td>
<td>RR 95% CI</td>
<td>Patients (n = 75) n (%)</td>
</tr>
<tr>
<td>RDS</td>
<td>74 (73)</td>
<td>55 (53)</td>
<td>1.4 (1.1-1.7)</td>
<td>40 (53)</td>
</tr>
<tr>
<td>Pulmonary air leak</td>
<td>18 (18)</td>
<td>5 (5)</td>
<td>3.6 (1.4-9.4)</td>
<td>13 (17)</td>
</tr>
<tr>
<td>Pulmonary hypoplasia</td>
<td>0</td>
<td>0</td>
<td></td>
<td>9 (12)</td>
</tr>
<tr>
<td>BPD at 28 days</td>
<td>31 (36)</td>
<td>19 (20)</td>
<td>1.9 (1.1-3.0)</td>
<td>25 (33)</td>
</tr>
<tr>
<td>at term</td>
<td>18 (18)</td>
<td>5 (5)</td>
<td>4.2 (1.6-11)</td>
<td>17 (23)</td>
</tr>
<tr>
<td>Infection by day one</td>
<td>1 (1)</td>
<td>11 (11)</td>
<td>0.1(0.01-0.7)</td>
<td>16 (21)</td>
</tr>
<tr>
<td>IVH Grades 3&amp;4</td>
<td>6 (6)</td>
<td>9 (9)</td>
<td>0.7 (0.2-1.8)</td>
<td>4 (5)</td>
</tr>
<tr>
<td>PVL</td>
<td>3 (3)</td>
<td>9 (9)</td>
<td>0.3 (0.1-1.2)</td>
<td>9 (12)</td>
</tr>
<tr>
<td>Primary hospital stay (days)</td>
<td>44</td>
<td>40</td>
<td>NS</td>
<td>46</td>
</tr>
<tr>
<td>median &amp; range</td>
<td>0-304</td>
<td>0-151</td>
<td></td>
<td>0-238</td>
</tr>
</tbody>
</table>

RDS = respiratory distress syndrome, BPD = bronchopulmonary dysplasia, IVH = intraventricular hemorrhagia, PVL = periventricular leukomalacia, RR = relative risk, OR = odds ratio
Table 5. Pulmonary outcome and growth at one year of corrected age in studies IV and V.

<table>
<thead>
<tr>
<th>Long-term outcome</th>
<th>Study IV</th>
<th></th>
<th>Study V</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients (n = 81)</td>
<td>Controls (n = 94)</td>
<td>RR (95% CI)</td>
<td>Patients (n = 55)</td>
<td>Controls (n = 56)</td>
</tr>
<tr>
<td>Pulmonary outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLD</td>
<td>12 (15)</td>
<td>3 (3)</td>
<td>4.6 (1.4-16)</td>
<td>12 (22)</td>
<td>5 (9)</td>
</tr>
<tr>
<td>steroids</td>
<td>14 (17)</td>
<td>4 (4)</td>
<td>4.1 (1.4-12)</td>
<td>10 (18)</td>
<td>6 (11)</td>
</tr>
<tr>
<td>median duration of rehospitalization (days)</td>
<td>0 (0-75)</td>
<td>0 (0-11)</td>
<td>0.04 #</td>
<td>0 (0-52)</td>
<td>0 (0-15)</td>
</tr>
<tr>
<td>Growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weight**</td>
<td>-4.1 (8.1)</td>
<td>-1.4 (8.3)</td>
<td>-5.3 - -0.3</td>
<td>-1.7 (9.2)</td>
<td>-1.0 (9.7)</td>
</tr>
<tr>
<td>length*</td>
<td>-1.2 (1.4)</td>
<td>-0.6 (1.2)</td>
<td>-1.0 - -0.2</td>
<td>-1.1 (1.3)</td>
<td>-0.9 (1.6)</td>
</tr>
<tr>
<td>head size*</td>
<td>-0.4 (1.1)</td>
<td>0.003(1.0)</td>
<td>-0.7 - -0.3</td>
<td>-0.3 (1.3)</td>
<td>-0.2 (1.1)</td>
</tr>
</tbody>
</table>

CLD = chronic lung disease, # = p-value, * as standard deviations of normal growth, ** relative to length as percentage of normal
6 Discussion

Preterm birth is one of the most devastating problems in perinatology, and predictive methods could help to identify those in whom therapeutic and surveillance measures should be introduced. The present study was established in order to investigate the impact on antimicrobial treatment of pregnant women with BV on the preterm birth rate. Particularly, a low risk population was targeted in screening for BV. Infectious etiological causes and biochemical markers have been evaluated earlier, but none are sufficient sensitive or specific in prediction of preterm birth. Because of the multifactorial etiology of preterm birth it seems reasonable to combine different markers, and calculate if such a test "battery" adds predictive value as regards of preterm birth.

A further aim was to evaluate the neonatal and long-term consequences on infants of indicated preterm birth, the incidence of which is increasing, and to see the peri- and neonatal, and long-term consequences of another important etiological cause of preterm birth – PPROM. Indicated preterm birth is a difficult problem for an obstetrician, who must balance intrauterine fetal hazards against the risks of prematurity. These decisions in the present study were aided by modern surveillance technology, such as CTG, and different ultrasonographic methods, especially Doppler ultrasonography. In this evaluation, special checking was directed to the cases with the worst Doppler findings in the umbilical artery (AEDV, REDV). PPROM has aroused the interest of researchers because of associations found to subclinical infections, and biochemical mediators such as cytokines. It can also cause pulmonary hypoplasia in the fetus, and may have a permanent effect on pulmonary development.

6.1 Risk of preterm birth in BV pregnancy: Effect of vaginal clindamycin (I)

In the present low-risk population the prevalence of bacterial vaginosis was much lower than expected. A much higher prevalence of BV (21%) was seen in the low-risk material of Kurki et al. (Kurki et al. 1992). This discrepancy is difficult to explain, since the same
Gram staining method was used and it is repeatable. Moreover, two thirds of the population participated in screening during the study period. This could even have reduced the possible bias of patient selection, and thus the found prevalence could be even lower. One explanation is that there could be some intrinsic fluctuation in BV at different times. The multiparous population in northern Finland may have different vaginal hormonal ecosystem, partly due to the less common use of contraceptive pills. In high-risk populations there could also be some additional anamnestic risk factors associated with BV, which could be a sign of another underlying pathological condition. On the other hand, BV has been reported to be an independent risk factor for preterm birth in a large study of over 10,000 women (Hauth et al. 1995) among whom confounding previous preterm deliveries were tested with regression analysis. Compliance problems are also always present in a study like this where the blinding of the test results is not possible- and patients want to have “proper” treatment.

Bacterial vaginosis appeared to increase the risk of preterm birth in the present study two-fold, as also found in other studies (Riduan et al. 1993, Meis et al. 1995), but not as much as at the study by Kurki et al., where the increase in preterm birth rate was 6.9-fold (Kurki et al. 1992). The increased risk of postpartal endometritis and CS wound infection in the present study is also in accordance with the results of previous studies (McGregor & French 2000).

The persistence of BV was seen more often in preterm deliveries than in term ones. Maybe more aggressive forms of BV have penetrated to deeper cervical tissues in early pregnancy, unreachable by local treatment in these cases. It is possible that BV itself can perhaps be retreated, but if choriodicidal damage has already occurred, the treatment – either by using systemic or local medication – may not be beneficial as regards reducing the preterm birth rate. The male partners of the BV women were not checked in the present study, because current data do not support routine treatment of male partners of BV women (Joesoef & Schmid 1995). Reinfections from sexual intercourse could, however, be an explanation in some cases of recurrent BV. Some of the women received other additional antibiotics for BV within the study protocol, but this was found not to be a confounding factor according to regression analysis. Recurrences, where BV was first cured and then reappeared, may also play a role in increasing the risk of preterm birth (two such cases in the present material), although in the present series the sample size is too small to draw any conclusions. Secondly, the spontaneous resolution rate of BV is very high (Hay et al. 1994), and it might be that some of the women with a BV+ finding at screening could in fact be already resolved at the time of randomization. The intermediate findings comprised an important subgroup that may reflect a continuum of vaginal floral change from normal to BV. Although two preterm births occurred in this group in the present series the proportion of BV+/− cases was far too small to make definite conclusions on the role of this subcategory.

According to the present results, vaginal clindamycin did not reduce the preterm birth rate or puerperal infections in a low-risk material. During the years of this study the results of other studies on BV have been published, with very variable results. Depending on whether the population was low- or high-risk, and on local or systemic treatment, different effects on pregnancy outcome have been noted. Local treatment with clindamycin has not been found to be effective in reducing the preterm birth rate in a high-risk population (Vermeulen & Bruinse 1999); instead it increased neonatal
infections. Growth of other potentially harmful flora, such as \textit{E. coli}, can be seen after clindamycin treatment (Hillier \textit{et al.} 1990). In addition, in a low-risk population using vaginal clindamycin, no reduction in preterm birth rate occurred (Joesoef \textit{et al.} 1995), as in the present study. The recruitment of the patients in that study was at relatively late weeks of gestation in some cases (14–26 weeks). In the present study, women were screened at 12 weeks of gestation and treatment was administered at a mean of 16 weeks, which could also have been too late. There is evidence that BV might affect the uterus, with marks of chronic plasma cell endometritis even before conception (Korn \textit{et al.} 1995). It should also be noted that medical treatment in early pregnancy always also involves a question of safety. Peroral treatment can be more effective, although its efficacy is seen only in high-risk material as regards reducing preterm birth rate (Hauth \textit{et al.} 1995). It is still difficult to come to a consensus, since the studies are so heterogeneous with varying sample sizes and study protocols. With the help of meta-analysis these results can, however, to some extent be combined to create conclusive results.

The present study was carried out in a relatively large screened population, but since the incidence of the main outcome, preterm birth, was low, the estimated sample size was not achieved. In the original power calculations we estimated that the present study needed to have 80% power to detect a significance of 0.05. With the detected prevalence of BV and rate of preterm births, however, the required sample size would have been over 4000. In addition, most cases of preterm birth were at near 36 weeks of gestation, and thus the really problematic preterm weeks of gestation were not represented.

The persistence of BV was clearly shown to increase the risk of preterm birth and puerperal infectious morbidity, according to the present results. The treatment of BV with vaginal clindamycin in low-risk pregnancy seems, however, not to be effective in reducing the preterm birth rate or infections in the puerperium. A third of cases of BV spontaneously recover, and maybe those not spontaneously cured represent more aggressive subgroups of BV. How to identify them is one of the future goals. Continuing research imperatives also include evaluating possible pre-pregnancy BV.

### 6.2 Prediction of preterm birth by means of cervical IL-6, IL-8, IGFBP-1, cervical ultrasonography and their combinations (II)

Elevated cervical IL-6 concentrations appeared to be the best predictor of preterm birth as a univariate parameter in this material, having, at a cut-off level of 128 ng/L, a specificity of 77% and a sensitivity of 73%. IL-6 has been studied extensively in recent years. The findings of the present study are consistent with those of a study with a chosen IL-6 cut-off level of 250 ng/L, predicting preterm birth with 50% sensitivity and 85% specificity (Lockwood \textit{et al.} 1994). The association between elevated IL-6 concentrations and preterm birth is understandable, since cervical IL-6 has also been found to identify MIAC in patients with preterm labor and intact membranes (Rizzo \textit{et al.} 1996), and MIAC is associated at risk of preterm birth. The elevated cervical IL-6 concentrations in the present study were not significantly associated with puerperal or neonatal infections. Neither did Lockwood \textit{et al.} find a correlation between endometritis and elevated IL-6 (Lockwood \textit{et al.} 1994). On the other hand, an association between maternal serum IL-6
and neonatal infections has been found in PPROM cases (Pfeiffer et al. 1999). The temporal relationship is not always clear – maybe ascending cervical infection is a primary risk factor – or intrauterine factors can initiate preterm birth. The concept of fetal inflammatory response has aroused interest recently (Gomez et al. 1998) as a potential independent fetal factor on the etiology of preterm birth. In cases of PPROM, IL-6 concentrations in fetal plasma obtained from cordocentesis seem to be higher in cases with a short interval between that procedure and delivery. No such association was found in amniotic fluid levels of IL-6 (Romero et al. 1998).

Concentrations of IL-8 (cut-off 4250 ng/L) also predicted preterm birth in the present study, with the same sensitivity as IL-6, but with a lower specificity (65%). IL-8 was elevated in cases of preterm labor (cut-off 1057 ng/L) with a sensitivity of 90% and a specificity of 95% in a study where preterm birth was not the outcome measure (Tanaka et al. 1999). IL-8 seems to appear after IL-6 in the cytokine network in cultured gestational tissues (Dudley et al. 1993). Cervical IL-8 may also predict intrauterine infection in patients with preterm labor and intact membranes (Rizzo et al. 1998), but in the present study no such correlation was found. The difference could be explained by the higher rate of clinical chorioamnionitis and the more advanced dilatation status of the cervix in the study by Rizzo et al. (Rizzo et al. 1998).

Elevated concentrations of cervical IGFBP-1 predicted preterm birth in the present study as univariate parameter, with 84% specificity and 45% sensitivity. IGFBP-1 measured in the present study was based on the phosphorylated isoform of IGFBP-1, secreted by the decidual cells. It is different from the nonphosphorylated isoform of IGFBP-1 present in the amniotic fluid, fetal serum and maternal plasma (used as a diagnostic test in amniotic fluid escape). In a study where cervical ripeness was investigated, involving both nonphosphorylated and phosphorylated isoforms of IGFBP-1, and resulting in the same 50% sensitivity, the value of the phosphoisoform was four-fold compared with that of the nonphosphorylated isoform. The phosphorylation status of cervical IGFBP-1 has been observed to be higher in late pregnancy decidua (Westwood et al. 1994). In the present material, preterm weeks of gestation were studied, where nonphosphorylated IGFBP-1 is the predominant form (Martina et al. 1997). Perhaps this explains to some degree the low concentrations in the present study with earlier gestation, compared with term pregnancies (Nuutila et al. 1999). Parallel samples for IL-6 and IL-8 were taken, and this theoretically might have led to lower concentrations of both in the buffer. The buffer amount used was 0.5 ml in the present study, where a 6.6 µg/L concentration cut-off was determined as being best. Careful rinsing of the swab is crucial in order to extract the specimen. The problem with these cervical markers, however, is high fluctuations in vaginal exudates causing a wide range of concentrations of the markers. Concentrations of serum markers would be more repeatable. Nevertheless, significant predictive values as regards preterm birth and neonatal infections were found. IGFBP-1 was the best marker as regards neonatal infectious morbidity, which was a novel finding. In the present study it was found that elevated levels of IGFBP-1 also predicted puerperal infectious morbidity, in line with other results (Kekki et al. 1999).

A cervical length cut-off of point of 29 mm was the same in this study as in that by Taipale et al. (Taipale & Hiilesmaa 1998), but it had higher sensitivity (52% vs. 19%), with a specificity of 82% vs. 97%. In the present study the gestational weeks at measurement were greater (over 23 weeks compared with 18–20 weeks in the study by
Taipale et al.) as was the prevalence of preterm birth (9% vs. 2%). The absolute length of the cervix shortens after 18 weeks of gestation (Okitsu et al. 1992). Nearly 3000 women were included in a study where cervical length seems to have a continuum, since cervical lengths below the 25th (30 mm), 50th (35 mm) and 75th (40 mm) percentiles were associated with an increasing risk of prematurity (Iams et al. 1996). Funneling in our study had a specificity of the same magnitude (70% vs. 74%) as in a study by Timor-Tritsch (Timor-Tritsch et al. 1996), but with much lower sensitivity (64% vs. 100%). The prevalence of preterm birth was nearly two times higher in that study. Determinations of funneling vary in different studies; both V- and U-shaped funnels can be included (Timor-Tritsch et al. 1996), as in the present study, or V-shaped funnels only (Gomez et al. 1994). The cervico-vesical fold helps in determining the upper boundary of the funnel (Shalev et al. 1999), the margins of which are not always clearly seen in U-shaped funnels. The funnel is not always present without pressure (Guzman et al. 1994), and the best cut-off values were also found in cervical parameters in the present study during times of transfundal pressure. The calculated cervical index reflects the funneling and cervical length was the best ultrasonographic marker in the present study as well as in a study of Gomez et al. (Gomez et al. 1994). The cut-off index value (0.52) in that study was higher than in the present study (0.20), but the cervixes were shorter (median 22 mm vs. 27 mm). To gain minimum intra-observer variability in measuring the cervix by ultrasonography the maternal bladder should be empty and the cervical canal must be fully identified sagittally so that the internal os and the external os are both visualized. Since the endocervical canal often curves, the true length is measured by combining straight distances, or by using an area trace method. In the present study, however, only the straight length was measured. Hence the measurement technique may potentially have some effect on the results.

It is clear that only some preterm births can be explained by cervical infection, in which these markers can be a sign and thus be of predictive value. In this context it also should be stressed that sometimes in cases of infection, preterm birth is the natural safety mechanism, which should not be stopped. The diagnosis of chorioamnionitis is often subclinical. Most upper genital tract infections remain asymptomatic and are not associated with fever (Goldenberg et al. 2000).

The possible clinical applicability of predictive markers also depends on the noninvasiveness of these tests, compared, for example, with amniotic fluid examination. There is also the question of cost, if numerous follow-up examinations are needed in prediction. The predictive value, on the other hand, applies to a relatively short time period, and the prediction interval varies (Leitich et al. 1999). In this respect an advantage of the present study protocol was in its being longitudinal. By having controls, some potential confounding factors that could explain the risk of preterm birth could be excluded.

The biochemical markers used, and ultrasonographic cervical changes as single tests, or in combination, are of predictive value as regards preterm birth. Even with relatively low sensitivities these test combinations provide an additional tool for making clinical decisions in surveillance and treatment options.
6.3 Clinical significance of AREDV in the umbilical artery in high risk pregnancy before the 34th week of gestation (III)

Doppler ultrasonography provides a valuable additional method for fetal surveillance in high-risk pregnancy. However, the individual timing of delivery remains problematic, e.g. because of the widely variable interval between the detection of AEDV and CTG pathology (Arabin et al. 1988, Jouppila & Kirkinen 1989, Arduini et al. 1993). The Doppler findings occur earlier than CTG pathology, the interval being 1–37 days in the present study. The total duration of AREDV is often unknown. Even in the present retrospective material it was impossible to know how long AREDV had already been present before its first detection. The interval between AREDV detection and delivery was one week in the present study, but with a wide range (1–52 days). A similar timing of delivery has been reported in the study by Karsdorp et al. (Karsdorp et al. 1994).

Hypertensive disorders were frequent in the present study. In a large multicenter study involving 245 AREDV pregnancies and 214 pregnancies without AREDV (Karsdorp et al. 1994) the proportion of cases of hypertension was 45% compared with 77% in our study. The figures are not quite comparable, because the occurrence of hypertension in AREDV women was not accurately reported in the multicenter study. IUGR of the infant was seen in 69% of the whole material in that study, compared with 82% in the present study. In 96 AEDV pregnancies (Trudinger et al. 1991) a PNM rate of 20.6% was found, which was of similar magnitude as in the present study. The condition of REDV infants was poorer than that of AEDV ones in the present study according to Apgar scores below 7 at one minute (77% vs. 40%). The finding is somewhat different from that in a study where a 68% rate of low Apgar scores at one minute was seen in AEDV material (Trudinger et al. 1991). The limit of low Apgar scores was set even lower than in the present study (6 vs. 7). The more important five-minute Apgar scores were, however, similar (27% low scores) in both studies in AEDV cases.

In a study by Mandruzzato et al. REDV has been associated with a 63% PNM rate and assessed as an indication for immediate delivery unless extreme prematurity is present (Mandruzzato et al. 1991). The differences in PNM rate cannot be explained by GA at delivery, which was also the same (31 weeks) in the present study, but the material was much smaller in the former study compared with the present study (32 vs. 83 cases). Overall mortality seems to be higher, the higher the frequency of REDV findings (Karsdorp et al. 1994). REDV was seen in 22% of the cases in the present study. Infants born after hypertensive pregnancies in the present study had lower PNM rates compared with the rest of the group. Maybe a lower frequency of anomalies among the hypertensive group cases compared with the rest of the material (9% vs. 37%) partly explains the difference. There were no differences in the PNM rate according to whether or not the indication for delivery was fetal or maternal.

The most frequent cause of death after exclusion of anomalies in the present study was RDS – not asphyxia. It can thus be asked whether the timing was too early. It obviously was not, because in early gestational weeks, the asphyxiated fetus would be in an even worse condition. The definition of asphyxia is, however, difficult and variable- generally it is referred as lack of oxygen and hypercarbia and metabolic acidosis. Asphyxia is even more difficult to diagnose antenatally, but maybe future diagnostic methods and their
combinations could provide better indicators for fetal hypoxemia (Vora & Gruslin 1998). It should be noted, that it is difficult and ethically impossible to carry out randomized prospective studies on this subject (Pattison et al. 1994).

Gestational week limits (23–34) were chosen in the present study on the basis of the viability of the fetus, and because of the highest neonatal risks occurring at below 34 weeks of gestation. In a larger study of over 160 high-risk pregnancies at between 31–36 weeks there were only ten cases of AEDV (Rochelson et al. 1987). The relatively large material in the present study compared with some other studies (Arabin et al. 1988, Fairlie et al. 1991) was collected during a seven-year period because of the relative rarereness of the finding.

A finding of AEDV, which precedes the ultimate REDV, has been shown to carry a risk of fetal mortality and also permanent brain damage (Valcamonico et al. 1994). An increased risk of IVH of the newborns was observed in a study by Karsdorp et al. (Karsdorp et al. 1994). Brain injury was speculated to be caused by alterations in cerebral flow redistribution. In the present study no systematic examination of fetal cerebral circulation was performed, nor was an increased frequency of IVH noted.

According to recent information, umbilical artery AREDV is a warning signal, after detection of which other Doppler ultrasonographic measurements, such as fetal venous (Kiserud 2000) and aortic arch circulation (Fouron et al. 1999) are required to identify fetal state more accurately.

6.4 Preterm birth for maternal or fetal indications (IV)

Indicated preterm birth has become an important cause of prematurity with an increasing frequency (Olsen et al. 1995). Because of the ethical aspects, a prospective or randomized study protocol could not be used in this situation, where threatened fetal asphyxia and prematurity are weighed against each other, and the clinical decision-making being problematic even with the benefits of modern fetal surveillance methods such as Doppler ultrasonography. In the present study, a matched control cohort study protocol was used. Cesarean section was carried out in all indicated cases in the present study, but it is difficult to find vaginal delivery counterparts in this kind of emergency situation. The obstetric decision to carry out preterm delivery in the early gestational weeks can have short-term consequences as regards neonatal mortality and morbidity and also long-term ramifications, that are seen later in life. A special hypothesis was that the long-term pulmonary outcome would be different in the indicated group compared with the control group, because of intrauterine stress factors in the forms of oligohydranmios and asphyxia in the study group.

The mothers were older in the indicated group, and thus hypertensive disorders more frequent, but age was revealed not to be a confounding factor according to regression analysis in the present study. Somewhat similar findings were observed in a study where older age of the mother was associated at the risk of indicated preterm birth (Meis et al. 1995). On the other hand, spontaneous preterm birth was associated with young maternal age according to multivariate analysis in that study. Early pregnancy bleeding was found to be a risk factor for preterm birth in both indicated and spontaneous groups at the study.
by Meis et al. (Meis et al. 1998). A contrary finding was seen in the present study, where first trimester bleeding tended to occur more often in cases of spontaneous preterm birth than in indicated ones. Maybe the histopathological placental infections that were more common in the spontaneous group (37%) than in the indicated (5%) group explain this early disruption of choriodecidua with subsequent bleeding. The higher rate of previous spontaneous abortions seen in the indicated group of mothers in the present study is difficult to explain. Perhaps spontaneous abortion can have similarities to an early trophoblast invasion defect, that can lead either to early abortion, or develop later into a hypertensive disorder exposing the mother to indicated delivery. A history of previous spontaneous abortions also increased the risk of indicated preterm birth according to a cohort study by Olsen et al. (Olsen et al. 1995).

Half of the indicated fetuses in the present study had an AREDV finding with reflections in neonatal parameters. Sometimes maternal and fetal indications were difficult to separate, but a fetal one was the main reason for delivery (61%) in the present study. The infants in the indicated group were in poorer condition at birth than their counterparts, which was not unexpected, because of the high-risk nature of the pregnancies.

Neonatal pulmonary problems were more frequent in indicated infants, which was reflected in higher neonatal morbidity and mortality. According to a study by Wolf et al. (Wolf et al. 1993), predischarge survival was not influenced by the primary complication that led to preterm delivery. They found, however, that hypertensive disorders were associated with an increased frequency of RDS, a finding which is in accordance with the present study. On the other hand, it has been observed that in very low birth weight (< 800 g) infants, there were no differences in the frequency of RDS among different etiological groups of prematurity (Iannucci et al. 1996). Lung maturation is affected by various factors, either enhancing or delaying. In high-risk pregnancies the infants are more stressed in utero which can have a maturational effect on the fetal lung, but at birth the infants can be asphyxiated and the pulmonary surfactant can be inactivated (Hallman et al. 1991). Oligohydramnios in these cases can physically restrict lung growth (Nimrod et al. 1984). It was present significantly more often in the indicated group than in the spontaneous group, partly explaining the pulmonary consequences. Infants born by CS also lack the lung compressive effect. AREDV infants in the indicated group appeared to have less RDS (although not significantly), as did those infants born after fetal indications, facts that suggest, if true, a maturational effect of intrauterine stress on the fetal lungs.

Pulmonary problems affect not only the neonatal period but also continue to at least one year of age, where the present study follow-up ends. Chronic lung disease was more common in indicated group infants at one year of age compared with their spontaneous counterparts. It occurred more often in the AREDV group than in the non-AREDV group in the present study. There might therefore be some delaying intrauterine factors associated with threatened fetal asphyxia that overcome the maturational factors. This can be harmful to the immature lung. Prenatal steroids have a maturational effect on the lungs (Crowley 1995). In the present study infants who received steroids had even more CLD, but maybe steroids were administered more often in the most highest risk cases. The other possibility is that although corticosteroids decrease the incidence of RDS, they can reduce lung growth, as has been suggested but not proven conclusively (Halliday 1999).
In the present material hypoglycemia and growth restriction were more common in the malnourished infants, with long-term effects on growth potential. The indicated infants were smaller in all growth parameters than their spontaneous counterparts. This can be explained by lower intrauterine energy reserves leading to hypoglycemia. The incidence of hypoglycemia in the present study was similar (over 70%) to that in a study by Duvanel et al. (Duvanel et al. 1999). With modern treatment strategies in which glucose infusion is administered immediately after birth, hypoglycemia was only transient and asymptomatic, thus not causing severe symptoms in the neonate.

In conclusion, it can be said that indicated preterm delivery is mainly a consequence of hypertensive disorders in the mother causing threatened intrauterine fetal asphyxia. Neurologic development of the offspring was, however, similar at one year of age in indicated as in spontaneous group infants, showing roughly that timing of the delivery may have been optimal in this material. Nevertheless the growth pattern of the infants is restricted and pulmonary function is impaired.

6.5 Consequences of PPROM on peri- and neonatal and long-term outcome of infants (V)

Preterm premature rupture of membranes is one of the causes of preterm delivery with special consequences of oligohydramnios (Nimrod et al. 1984) and a high frequency of infections in the fetus. The long-term pulmonary ramifications of PPROM have been observed in some relatively small studies (Beydoun & Yasin 1986, Morales & Talley 1993). Active expectant management prolonging pregnancy in PPROM cases, with the use of tocolytics and antibiotics, has resulted in good neonatal outcomes (Fortunato et al. 1994).

The infants in the present study were investigated from 17 until 30 gestational weeks. In addition, some very early cases in which PPROM occurred after genetic amniocentesis after 16 weeks were included the present study. Follow-up data on nearly all the cases was obtained.

The significantly more frequent previous cervical operations in PPROM patients were understandable, because of the trauma causing weakening of the cervical tissue.

Antepartum bleeding was detected significantly more often in PPROM cases than in controls, and can provide an infection route. This has also been observed by Harger et al. (Harger et al. 1990). A route of infection opens when choriodecidual detachment occurs. PPROM has also been found to be connected to placental abruption (Major et al. 1995). One theory concerning this involves the mechanism of uterine decompression and surface area disproportion after PPROM, and oligohydramnios status. Episodes of bleeding can also weaken the membranes by compromising their nutritive support, eventually leading to PPROM and ultimately to abruptio placentae.

It is surprising that the rate of clinical amnionitis was not higher in PPROM cases than in controls. Maybe those excluded cases with delivery after PPROM within 24 hours had had more infections. The frequency of histopathological chorioamnionitis was significantly higher in PPROM cases than in controls, which is in accordance with the findings of Arias et al. (Arias et al. 1997). The underlying and often subclinical infection
is thus clearly documented. It is noteworthy, because current practice is to administer prophylactic antibiotics in PROM cases (Mercer & Arheart 1995). Antibiotic prophylaxis was, however, not frequent at the time of this study. Puerperal infections, which also were more frequent in PPROM patients compared with controls, can be explained by CS wound infections and also by underlying intrauterine infection.

The CS rate was higher in the PPROM group than in the spontaneous group. The reason for CS in PPROM cases is threatened asphyxia and presentation abnormalities of the fetus. Breech presentations were, however, not registered in the present study.

Oligohydramnios can cause hypoplasia of the lungs, other organs and limbs. The latency period to delivery in the present study was longer (mean 31 vs. 9 days) in cases where PPROM occurred earlier (before 25 weeks). Pulmonary hypoplasia in PPROM infants was more common in cases where the interval from PPROM to delivery was over 31 days, according to the findings of the present study. Hence this complication seemed to need a relatively long period to develop. The frequency of lung hypoplasia was 12% in the present study compared with 5% in an earlier study (Morales & Talley 1993). This difference cannot be explained by fewer gestational weeks (PPROM before 25 weeks) in that study, because of an even longer latency period before delivery (10 days) than in our study (7 days). Maybe definitions of pulmonary hypoplasia differ in these studies. With modern treatment, the cases with lung hypoplasia, that previously were lost, could nowadays be treated and survive, but the infants may have CLD. The infants in PPROM group still required more rehospitalization days because of lung problems.

Infants born after PPROM in the present study were smaller, in accordance with earlier findings (Hediger et al. 1995). This can be explained by diminished nutrition caused by oligohydramnios and compression. Limb contractures were found less often in the present cases, a figure which is lower than in other studies (McIntosh & Harrison 1994). The latency period before delivery was, however, shorter in the present study.

At one year of age, catch-up growth in PPROM infants had occurred, indicating growth potential. In the present study no differences in neurologic outcome were noticed between the study and control groups. An opposite result has been found in a previous study, showing an increased frequency of CP in infants born after PPROM (Murphy et al. 1995). An increased rate of infection may also cause tissue damage at the level of the brain. This has been noticed in connection with PVL, in which one etiological cause is thought to be infections (Spinillo et al. 1995). An explanation for the differences in neurological outcome may be that the rates of neonatal infection did not differ in the present study between the groups.

Indicated preterm delivery as well as delivery after PPROM compared with spontaneously preterm born infants, can lead to detrimental pulmonary effects, although the mechanisms in these groups can differ. Growth “programming” can occur *in utero* in these premature cases, and according to Barker’s hypothesis, it may have lifelong ramifications in adult disease.
7 Conclusions

The following conclusions can be drawn from the results of the present study:

1. The prevalence of bacterial vaginosis in early pregnancy in a low-risk population was low (only 7.3%). Persistence of BV increased the risk of preterm birth and puerperal infectious morbidity. Vaginal clindamycin treatment seemed to be ineffective in reducing the rate of preterm birth or puerperal infection in low-risk patients with early pregnancy BV. Spontaneous resolution of BV seems to occur frequently. Because of the small sample size, conclusive remarks cannot be made.

2. Cervical IL-6 (> 128 ng/L), IL-8 (> 4250 ng/L) and a cervical index > 0.2 as univariate markers appear to have sensitivities and specificities of similar magnitude (approximately 70%) in predicting preterm birth. Elevated cervical IGFBP-1 appears to have the best specificity, with a relatively low sensitivity. Cervical index was superior to the cervical ultrasonographic parameters. The combination of cervical index and IL-6, or cervical index and IGFBP-1, seems to be highly specific in predicting preterm birth, but the sensitivity remains low. IGFBP-1 appeared to be a significant marker of neonatal and puerperal infections. The relatively low sensitivity limits the clinical applicability of these markers.

3. Maternal hypertensive disorders and IUGR appear to be characteristic in AREDV pregnancies. The interval between AREDV detection and the first pathological CTG finding is highly variable (1–37 days). An AREDV finding in the umbilical artery is a warning signal of fetal asphyxia in high-risk pregnancies. It was reflected in a PNM rate of 19% in the present material. The REDV cases had a higher PNM rate than the AEDV cases. The major cause of death of the infants was anomaly, followed by RDS.

4. Maternal hypertensive disorders and a finding of AREDV in umbilical artery indicating threatened fetal asphyxia were characteristic in indicated delivery. The NNM rate, pulmonary morbidity and hypoglycemia appeared to be more frequent in indicated delivery infants compared with their spontaneous counterparts. Chronic lung disease appeared to occur more often in indicated vs. control infants, and they also remained smaller than their counterparts at one year of corrected age.

5. Histologic chorioamnionitis is more common and characteristic in PPROM pregnancies compared with cases without PPROM. A relatively long latency period between amniotic fluid escape and birth (mean 31 days) was seen in cases with
pulmonary hypoplasia. The main cause of fetal or neonatal death in these pregnancies is infection followed by RDS. There appear to be no differences in neonatal mortality or morbidity between PPROM infants and non-PPROM counterparts. PPROM appears to cause a disturbance in lung development, increasing the possibility of later CLD, and thus it increases the need for rehospitalization, at least until up to one year of corrected age.
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