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ESTIMATION OF GESTATIONAL AGE BY ULTRASOUND

AND

EXTREME PREMATURITY

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ABSTRACT

Accurate estimation of the gestational age of the fetus is a key assessment made by providers of obstetric care during pregnancy, since decisions concerning management strategies are dependent on this estimate. Thus, the prognosis for preterm infants born at the border of viability is strongly dependent on the accuracy with which gestational age can be determined.
The aim of the present theses was to investigate the impact of maternal obesity, different procedures for dating and the different formulae employed in connection with ultrasonographic values on the estimation of gestational age. Furthermore, the incidence of and factors that influence the one-year survival of infants born extremely preterm were explored.
Our examination of the data from the EXPRESS study, which cover infants born prior to 27 weeks of gestation, revealed a one-year survival rate of 70%. The chance for survival without any major morbidity increased significantly with advancing gestational age at birth, from 9.8% at 22 weeks to 85% at 26 weeks of gestational age.
In accordance with current recommendations in Sweden, estimation of gestational age in 95% of the pregnancies included in the EXRPESS registry was based on measurements of biparietal diameter and femur length by routine ultrasound examination usually performed during mid-trimester. However, the applications of different procedures and dating formulae in other countries make comparisons of rates of neonatal mortality and morbidity both difficult and unreliable. Therefore, we examined estimation of GA based on the last menstrual period (LMP) in this same cohort. The predicted duration of pregnancy based on LMP was in general longer than when assessed by ultrasound, but the rates of survival and morbidity were the same with both approaches. Moreover, we found that despite the fact that the dating formulae developed by Hadlock, Persson and Mul and coworkers are all based on ultrasonographic measurements of biparietal diameter (BPD) and femur length (FL), the estimates of the gestational age that they provide for infants later born extremely preterm differed significantly. Fetuses which are found upon ultrasound examination to be at least 7 days smaller than expected on the basis of the LMP, exhibit an elevated risk for being born small for gestational age (SGA) as well as for stillbirth. In our extensive cohort study based on the Medical Birth Registry, the risk for such a discrepancy was found to be enhanced among obese mothers, increasing linearly with increasing maternal BMI. In this case, all of the dating formulae based on BPD and FL produced similar prediction of SGA.
In conclusion, the procedure employed, the choice of ultrasonographic formula applied, and maternal obesity, all influence assessment of gestational age. These findings should be taken into consideration in managing pregnancies that result in preterm infants born on the edge of viability.

LIST OF PUBLICATIONS

I. EXPRESS Group.

One-year survival of extremely preterm infants after active perinatal care in Sweden.

JAMA. 2009 Jun 3; 301(21):2225-33.

II. Simic M, Wåhlin IA, Maršál K, Källén K.

Maternal obesity is a potential source of error in mid-trimester ultrasound estimation of gestational age.

Ultrasound Obstet Gynecol. 2010 Jan; 35(1):48-53

III. Simic M, Maršál K, Amér-Wåhlin I, Källén K.

Differences in ultrasonically estimated gestational age of extremely preterm infants when using various dating formulae

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IV. Simic M, Amér-Wåhlin I, Lagercrantz H, Maršál K , Källén K.

Survival and neonatal morbidity among extremely preterm born infants in relation to gestational age based on the last menstrual period or ultrasonographic examination.

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CONTENTS

LIST OF ABBREVIATIONS

AD	Abdominal diameter
BMI	Body Mass Index
BPD	Biparietal diameter
CI	Confidence interval
EDD-LMP	Estimated date of delivery according to the last menstrual period
EDD-US	Estimated date of delivery according to ultrasound
EXPRESS	Extremely preterm born infants in Sweden
FL	Femur length
GA	Gestational age
GA-LMP	Gestational age according to last menstrual period
GA-US	Gestational age according to ultrasound
HC	Head circumference
IVH	Intraventricular hemorrhage
IUGR	Intrauterine growth restriction
LMP	Last menstrual period
MBR	Medical birth registry
NEC	Necrotizing enterocolitis
OR	Odds ratio
PPROM	Preterm premature rupture of membranes
cPVL	Cystic periventricular leukomalaci
ROP	Retinopathy of prematurity
SBU	Swedish Council on Technology in Health Care
SD	Standard deviation
SGA	Small for gestational age
WHO	World Health Organization

1 AIMS

The overall objective of this thesis was to study factors that influence assessment of gestational age based on ultrasound examination and the impact of such estimation of gestational age on infants born extremely preterm.

The specific aims were:

- to determine the one-year survival with and without major neonatal morbidity among infants born extremely preterm

- to investigate the influence of current perinatal interventions on neonatal survival of infants born extremely preterm

- to investigate the possible impact of maternal obesity on ultrasonographic dating of pregnancy

- to compare the gestational age estimates by three dating formulae applied to a cohort of extremely preterm born infants

-to investigate the potential impact of gestational age estimation on the basis of the last menstrual period in comparison with gestational age based on ultrasound examination, on rates of survival and neonatal morbidity among extremely preterm born infants.

2 INTRODUCTION

Establishment of an accurate “due date” for pregnant women is of both social and medical significance. The woman and her family plan various economic and social activities around this estimated and long awaited birth day of their child. [1, 2]. Providers of obstetric care use this end point date to schedule maternal and fetal testing during the pregnancy, gauge parameters of fetal growth, and apply timelines for specific interventions for the management of prenatal complications. Indeed, critical decisions concerning management of preterm labour, the timing of post date induction of labour and identification of intrauterine growth restriction are all based on the presumed gestational age of the fetus, as calculated backwards from the estimated day of delivery. [3, 4]

Moreover, for an infant born at the border of viability, treatment is adapted to his/her gestational age. Perinatal mortality and neonatal morbidity among infants born extremely preterm are strongly correlated to the gestational age at birth. In addition, calculation of the expected birth weight and thereby postnatal diagnosis of fetal growth restriction is based on the estimated gestational age at birth.

Ultrasonography during pregnancy is one of the technology methods most commonly used in health care, primarily due to its routine appliance in developed countries for estimation of gestational age, for which this approach is today considered to provide the most accurate value. However, the accuracy of this procedure depends greatly on the quality of the images obtained, which can be impaired by maternal obesity and position of the fetus.

Furthermore, there is still no general consensus on the optimal gestational age for ultrasonographic examination or on the formula employed to calculate gestational age. Thus, possible systematic errors within studies will cause variation in reported survival and neonatal morbidity.

One limitation is that ultrasound biometry is based on the presumption that fetuses of the same size at the time of ultrasound assessment, are also of the same age. If the fetus' growth is restricted it will be smaller than expected and the estimated date of delivery will be postponed relative to a term based on calculations established by last menstrual period. Such a discrepancy is not only indicative of early intrauterine growth restriction of the fetus but is also associated with adverse perinatal outcome.

In four studies included in this thesis, I focus primarily on methods for estimation of gestational age and their impact on the duration of pregnancy. My aim was to describe the current incidence of mortality and morbidity among extremely preterm born infants and to study the potential impact of two pregnancy dating procedures on the perinatal outcome. In addition, I examined the impact of maternal obesity and various ultrasonographic dating formulae for calculation of gestational age on the duration of pregnancy. The research presented in this thesis, performed in Sweden during the period of 2007-2012, is based on the Swedish Medical Birth Register and the Extremely Preterm infants in Sweden (EXPRESS) registry.

3 BACKGROUND

3.1 ESTIMATION OF GESTATIONAL AGE

Accurate dating of gestational age (GA) is one of the most important assessments performed during pregnancy, given that all of the various management strategies are dependent on knowing the gestational age of the pregnancy.
Before the ultrasound examination became the method of choice for estimation of GA, it was based on the records of last menstrual period.

3.1.1 Estimation of gestational age on the basis of last menstrual period

Normally, human gestation lasts for an average of 266 days from the date of conception or 280 days from the first day of the last menstrual period (LMP).[4, 5] Based on the assumption that a typical menstrual cycle lasts 28 days , with ovulation occurring on approximately day 14 the 19^{th} –century obstetrician, Franz Karl Naegele developed a simple calculation for estimated date of delivery that involved adding 9 months and 7 days to the first day of the LMP.[1, 6] This calculation, referred as Naegele`s rule, provides an indirect measure of the time of conception and remains the current standard for calculating the duration of pregnancy based on the LMP. [5, 7]

The reliability of this approach depends on a number of factors including the woman`s accurate recall of her LMP, the regularity of her menstrual cycles and possible use of contraceptives or breastfeeding that could influence the timing of ovulation. [3, 8, 9] Moreover, the actual timing of ovulation can also fluctuate [10-12] and it has been claimed that woman may become pregnant on any day of her menstrual cycle, including the first day. [13] Because of such potential errors, estimation of GA based on LMP is considered to be less reliable than ultrasonographic examination.

3.1.2 Estimation of gestational age based on ultrasound examination

Although diagnostic ultrasound examination during pregnancy was introduced into routine medical praxis in the 1970s,[14, 15] the Scottish physician Ian Donald, published the first scientific report on medical use of ultrasound entitled "Investigation of Abdominal Masses by Pulsed Ultrasound" in The Lancet as early as 1958.[16] Initially, such examinations during pregnancy were performed only in cases of a medical problem or in women at high-risk for pregnancy complications. However, since 1970s ultrasound screening has become routine in virtually all Western countries with a scan at 16-24 gestational weeks being employed primarily to confirm the viability of the fetus, date the pregnancy and detect multiple pregnancies.

3.1.2.1 The Physics of ultrasound

Ultrasonography is a sophisticated radiological method for location, measurement and delineation of deep structures by measuring the reflection of high frequency (ultrasonic) waves. A transducer moving across the area to be examined emits pulses of ultrasound, which propagate through the tissues. Some of the pulses are reflected back to the transducer which converts returning echoes into electric signals with the strength of the echo being determined by the characteristics of tissue interface. A computer displays both the strength and position of each echo as an image on a screen. Calculations of the distance to the sound reflecting surface plus the known orientation of the sound beam give a two- or three-dimensional image.[17]

Estimation of GA by ultrasound is based on measurement of one or more fetal biometric parameters. During the first trimester, mean diameter of the gestational sac and the crown-rump length (CRL) are parameters employed. [18-20] During the second and third trimesters, measurements of the fetal head (most commonly measures include biparietal diameter (BPD) [21] and head circumference (HC))[22], body (abdominal circumference (AC))[23] and extremity (femur length (FL)) [24-26] are commonly utilized to assess gestational age. Numerous other parameters have also been measured, but few improved the accuracy of assessment. [27]

The biometric values (expressed in millimeters) are subsequently converted into days with various so called dating formulae i.e. mathematical equations based on regression analysis that describe the curve of best fit for GA as a function of one or more fetal biometric parameters. [28, 29] Although, all of these formulae are constructed using values from women for whom highly reliable menstrual or conceptual dates are available i.e. these measurements are assumed to represent "true" GA, the standard population of women, the number of measurements, and GA at the time of examination involved vary. Most formulae utilized during the second trimester are based on the measurements of fetal head and of both fetal head and extremity, although Mongelli and co-workers have showed that combining two variables had no advantage over single-parameter formulae. [30] On contrary, Persson and Weldner and others have concluded that a combination of biparietal diameter and femur length provides the most accurate estimate of gestational age. [29, 31, 32].

3.1.2.2 Limitations of ultrasonographic examination

Safety of ultrasound examination during pregnancy

Since pulsed sound waves can raise the temperature of body tissues, the safety of ultrasound has been a matter of some concern. The potential harmful effects of such vibration, commonly referred to as cavitation, to the fetus [33, 34] have been examined in laboratory and epidemiological studies which have provided no clear evidence to date of any adverse effect of exposure to the low levels of ultrasound energy currently used on human fetuses. [33, 34] [35-38] However, in light of the possibility of unknown adverse effects, current guidelines state that ultrasound during pregnancy should be performed only for medical reasons and in accordance what is commonly referred to as the "ALARA" principle (as Low as Reasonably Achievable).[39] Moreover, the time for an examination should be minimized.[33]

The influence of maternal and fetal characteristics on ultrasound findings

Due to the increasing incidence of obesity, the body size of the patient is today probably the most common factor that interferes with the quality of the images and thereby, with the accuracy of an ultrasound examination.[33] Despite substantial technological improvements, the examination of obese patients remains a challenge due to the negative effect of adipose tissue on propagation of sound waves. The abdominal fat elevates the number of interfaces and causes marked attenuation of the signal impairing image quality, which is a consequence of absorption, reflection, reverberation, and scatter [40] Thus, maternal habitus may reduce the quality of anatomical scans as well as the accuracy of measurements required for estimation of GA. [41, 42]

The quality of ultrasound imaging also depends on the technical capabilities of the ultrasound equipment as well as on the experience and expertise of the operator. Furthermore, other variables such as gestational age and fetal position may also influence image clarity. [43]

Discrepancy between expected gestational age by LMP and estimated gestational age based on ultrasound examination

Although, ultrasound examination is considered to be more reliable than the use of certain menstrual history for predicting the date of spontaneous delivery, ultrasound dating does disregard biological variations in the rate of fetal growth and length of pregnancy. [44-46] Comparisons have revealed that, on average, ultrasound examination provides a younger estimate of gestational age than that calculated from the LMP. [46-49] This discrepancy is enhanced by young maternal age, lower maternal education, Hispanic ethnicity, unmarried status, cigarette smoking, primiparity, non-optimal BMI (< 18.9 or >29.0 kg/m2) and diabetes.[47, 48, 50, 51]

Fetuses that are smaller than expected upon ultrasound examination are overrepresented among infants weighing less than expected at birth a situation that may reflect early intrauterine growth restriction (IUGR).[52-55] Such fetuses also have a significantly elevated risk for perinatal death and preterm birth.[56, 57]

The current clinical practice of considering the due date estimated by ultrasound to be more accurate than that based on LMP, may incorrectly underestimate the gestational age of fetuses that are smaller than expected at ultrasound examination in mid-trimester. This can in turn hinder detection of early growth restriction. Such systematic inaccuracy in the dating of gestational age could distort correlations between maternal characteristics and adverse pregnancy outcomes (e.g. preterm birth) based on erroneous gestational age leading to misclassification.

3.1.2.3 The benefits of pregnancy dating by ultrasound examination

Although the use of ultrasound examination for, among other indications, estimation of gestational age has become routine in many developed countries during the past few decades, there is no evidence that this procedure has led to a general reduction in perinatal morbidity or mortality. [58, 59] The implication of ultrasound examination

has reduced the number of pregnancies judged to be post-term and thereby the corresponding rate of inductions for assumed post-term pregnancy.[60, 61] However, the perinatal outcome has not improved. [62] One of the most important studies in this area , was the Routine Antenatal Diagnostic Imaging with Ultrasound study (RADIUS), published in 1993, a multicenter, randomized controlled trial that examined the efficacy of routine ultrasound screening in more than 15 000 pregnant women. Routine ultrasound screening including estimation of gestational age during pregnancy did not influence the incidence of adverse perinatal outcomes, such as fetal death, neonatal death or neonatal morbidity. [62]

Despite the lack of scientific evidence, routine ultrasound examination during pregnancy including estimation of gestational age is nonetheless considered to be the golden standard for fetal assessment in current clinical practice.

3.1.3 Current practice in Sweden and internationally

The current recommendation in Sweden is to perform a routine ultrasound examination during the second trimester, in order to determine the number of fetuses present, to estimate the gestational age, and to locate the placenta. At the same time, this examination provides an opportunity to diagnose congenital anomalies as well as to identify maternal pelvic pathology.[63] Since the accuracy of the ultrasound examination depends to a considerable degree on the examiner and the quality of the images, technical and training issues have been addressed by the organization Swedish Technology Assessment in Health Care (SBU) [63] which has set professional standards for equipment specifications and training.

According to the SBU, during 1997, most departments of obstetrics in Sweden carried out ultrasound dating based on the BPD and FL measured at 16 – 20 postmenstrual weeks after the last menstrual period. [63] The Swedish Society for Obstetrics and Gynecology recommends that the fetal BPD should be measured from the outer edge of the proximal parietal bone to the inner edge of the distal parietal bone at the level of the thalami and cavum septi pellucidi. The FL should be measured with the ultrasound transducer positioned at an angle of 45˚to the bone. The dating formula most commonly employed in Sweden was developed by Persson and Weldner. [31]

Despite the evidence supporting the reliability and accuracy of ultrasound examination, the routine use of this procedure is not always recommended for all pregnancies internationally. For instance, in the United States, in the absence of maternal complications, gestational age estimation on basis of LMP remains the preferred method for pregnancy dating.[35, 36] The Canadian recommendations are similar to the Swedish guidelines, except for the measurement of head circumference (HC) and abdominal diameter (AD) that are also included.[64] Both the Australasian Society for Ultrasound in Medicine and British Royal College of Obstetrics and Gynecology [65] recommend that estimation of GA by ultrasound should be based on BPD, FL and HC.[66]

The method employed for determination of gestational age, the duration of pregnancy at which the ultrasound examination is performed as well as the measurement procedure, equipment and dating formula utilized may all influence the value obtained. [67, 68] Since a diagnosis of prematurity is determined by gestational age, such

variation reduces the reliability when comparing international data on preterm birth. Even in extensive international studies on extreme prematurity, the GA is defined primarily as the number of weeks of amenorrhea and the method utilized for GA estimation is not described in detail. [69-72]

3.2 EXTREME PREMATURITY

3.2.1 Definition

The WHO defines preterm birth as childbirth occurring after less than 37 complete weeks or 259 days of gestation. [73] Preterm births can be subdivided according to GA: approximately 5% occur at less than 28 weeks (extreme prematurity), 15 % at 28-31 weeks (severe prematurity), 20% at 32-33 weeks (moderate prematurity) and 60-70 % at 34-36 weeks (near term). [74]

Preterm births can also be subdivided according to the obstetrical precursors leading to preterm birth into indicated and spontaneous preterm births. [75] The obstetric precursors leading to preterm birth are: delivery for maternal or fetal indications, in which labor is either induced or the infant is delivered by prelabour caesarean section; spontaneous preterm labor with intact membranes; and preterm premature rupture of the membranes (PPROM), irrespective of whether delivery is vaginal or by CS. [76] About 25% of preterm births are indicated. [77, 78] Spontaneous preterm and PPROM labor account for another 25% and 50 % of all preterm births, respectively. [79]

The medical reasons for induction of preterm labour include severe maternal hypertension, ablatio placentae, or endangered fetal well-being, such as intrauterine growth retardation, or “fetal distress”. [78, 79]

Spontaneous preterm labor is defined as regular contractions accompanied by cervical alterations at less than 37 weeks of gestation. Preterm labor is now thought to be a syndrome with a pathogenesis that is not well understood, but that might involve early idiopathic activation of the normal labor process or to be the result of the pathological insults.[80] Preterm labor can be initiated by a variety of factors, including infection or inflammation, uteroplacental ischemia or hemorrhage, uterine over distension, stress and other immunologically mediated processes [81].

PPROM is defined as spontaneous rupture of the membranes at less than 37 weeks of gestation and at least one hour prior to the onset of contractions. In most cases the cause is unknown, but asymptomatic intrauterine infection is a frequent precursor and the other risk factors for PPROM are generally similar to those for preterm spontaneous labor with intact membranes [78]. Most women experiencing PPROM go into labour spontaneously within days, but a small proportion does not deliver until weeks or months later.

Many maternal and fetal features including demographic characteristics, nutritional status, previous pregnancy, obstetrical history and pregnancy characteristics, psychological factors, adverse behavior, infections, uterine contractions and cervical length, as well as biological and genetic markers have been associated with an elevated risk for preterm birth. [82, 83]

3.2.2 Incidence of preterm birth

Few countries, like France and Finland have reported a reduction in incidence of preterm births. Indeed, the United States have experienced a small but steady rise in the incidence since the early 1980s [84] whereas in Sweden, the incidence of preterm birth prior to 33 weeks of gestation has remained steady at 1.3% since the 1980s. This growing or unaltered incidence, despite the advances in perinatal medical care, might reflect increasing maternal age,[85] enhanced numbers of pregnancies resulting from assisted reproductive treatment [86] , and the introduction of new risk factors related to lifestyle [87] as well as the fact that statistics on preterm births now include cases that in the past were considered "late" abortions. Enhanced use of early ultrasound examination for estimation of gestational age or preterm delivery in cases of extreme fetal growth retardation may also contribute to increased incidence.[88]

3.2.3 Perinatal mortality and neonatal morbidity

During the past two decades, survival rates among infants born extremely preterm have increased substantially as a result of advances in knowledge, medical technology and therapeutic options. [69, 89-91] Unfortunately, this improved survival has not been accompanied by corresponding reductions in neonatal morbidity and rates of long-term morbidity remain high. [92-96]

Survival rates demonstrate a strong positive correlation to GA at time of birth. Thus, in recent population-based studies survival rates upon hospital discharge have been reported to be 0% with an age of 22 gestational weeks at birth, 6% - 26% at 23 weeks, and 29-55% at 24 weeks. [72, 94, 97] Other risk factors known to be associated with an elevated risk for adverse neonatal outcome among infants born extremely preterm include male sex, multiple pregnancies, SGA, and an Apgar score at 5 minutes of 3 or less. [62, 69, 98]

Although many extremely preterm born infants develop normally, neonatal morbidities such as neurological, ophthalmological, gastrointestinal or pulmonary damage. High grade intraventricular hemorrhage (IVH) (≥grade 3) [99], cystic periventricular leucomalacia (cPVL) (11), bronchopulmonary dysplasia (BPD) (12), retinopathy of prematurity (ROP) (13), necrotizing enterocolitis (NEC) [100], neonatal infection [101] and poor growth from the time of birth to discharge [102] are often antecedents of long-term devastating disabilities.

Intraventricular hemorrhage (IVH)

The brain disorder IVH occurs exclusively in preterm infants with a 20-30% incidence among those born at less than 31 weeks of GA.[103] In attempt to describe the varying degrees of IVH, Papile and colleagues [99] grouped the associated CT findings into 4 grades on the basis of the location of the haemorrhage: Grade 1, subependymal hemorrhage; Grade 2, intraventricular hemorrhage without ventricular dilatation; Grade 3, intraventricular hemorrhage with ventricular dilatation, and Grade 4, intraventricular hemorrhage with parenchymal hemorrhage. IVH correlates strongly to subsequent adverse neurodevelopment.[104, 105] Presumably, the more severe the grade, the greater the risk for associated neonatal morbidities and, in particular, adverse long-term

neurodevelopment which has an incidence of approximately 35% in grade 3 and as high as 90% in grade 4. [106]

Cystic periventricular leukomalacia (cPVL)

Cystic periventricular leukomalacia (cPVL) is characterized by necrosis in white matter located near the lateral ventricles in the brain. Cystic areas deep in brain white matter appear following hemorrhagic and ischemic infarction which. These irreversibly damaged areas appear as echo lucent cysts on neuroimaging studies. [103] cPVL is associated with a significantly elevated risk for cerebral paresis. [107, 108]

Bronchopulmonary dysplasia

Bronchopulmonary dysplasia (BPD) is a chronic lung disease of premature infants that affects approximately 20–30% of children born at a gestational age of less than 30 weeks and nearly 50% of those born at 26-28 weeks of gestation. Its development is related to pulmonary immaturity at the time of birth, exposure to high concentrations of oxygen and trauma caused by being on a ventilator. Fortunately, surfactant replacement therapy has resulted in a general diminution in the severity of the chronic lung disease. In the epidemiological studies, the strongest risk factors for BPD are low birth weight, followed by low gestational age [109]

A requirement for oxygen supplementation for at least 28 days after birth and at 36 weeks of postmenstrual age together with a need for positive airway pressure are used to categorize the severity of the disease as mild, moderate or severe [102]. The mortality rate is relatively low but there is still considerable morbidity. [110, 111] The severity of BPD is a strong predictor of abnormal pulmonary function and need for health care during childhood [112]. Moreover, the children affected are more likely to exhibit delay in the development of language, cerebral palsy, and cognitive impairments [113].

Necrotizing enterocolitis

Necrotizing enterocolitis (NEC), a bowel disorder of newborns is characterized by abdominal distention, ileus, and bloody stools. In addition, there is usually radiological evidence of pneumatosis intestinalis, (i.e. gas in the bowel wall that is produced by invading bacteria). Bowel perforation may prompt resection.

The pathogenesis of the disease involves multifactorial interactions between an immature gastrointestinal tract, mucosal injury, and potentially injurious factors present in the lumen. In as many as 20% of affected infants, the only risk factor is prematurity [114]. Treatment can be medical or surgical (if there is evidence of perforation); the mortality rate is 10% and the long-term prognosis is determined by the degree of intestinal loss.[115]

Retinopathy of prematurity

Retinopathy of prematurity (ROP) occurs only in the incompletely vascularized retina of the premature infant with the highest incidence in the infants with the lowest GA at birth. This condition appears to be triggered by some initial injury to the developing retinal vessels. It s severity is graded on the basis of the degree of abnormal vascular development and retinal detachment (I-V) as well as on the region of the eye affected (1-3).[116, 117]

Intrauterine growth restriction

Infants who weigh less than normal (below the 10th percentiles) for their sex and GA at birth are referred as small for gestational age (SGA). [118] In Sweden, a value of 2 standard deviations (SD) is defined as SGA. Some SGA infants are constitutionally small and still growing whereas others have experienced intrauterine growth restriction (IUGR). Early assessment of GA, as well as careful measurement of uterine fundal growth throughout the pregnancy, can help identify many cases of abnormal fetal growth, which can be caused by poor maternal environment, intrinsic fetal abnormalities, congenital infections or other forms of fetal malnutrition.

Fetal growth restriction is associated both with substantial perinatal morbidity e.g. birth asphyxia, meconium aspiration and neonatal hypoglycemia and hypothermia and abnormal neurological development, as well as elevated mortality. [119, 120] Moreover, the risk of long-term mortality is significantly increased for such infants. [121] The postnatal growth and development of the growth restricted fetus depend on the cause of restriction, the nutrition status during infancy, and the social environment. [122] With growth restriction due to congenital, viral or chromosomal factors or maternal size the individual usually remains small throughout life whereas when growth restriction is due to placental insufficiency, infants most often exhibit “catch-up” growth and approach their inherited growth potential.

3.2.4 Perinatal factors associated with mortality and neonatal morbidity

Rising rates of neonatal survival among infants born extremely preterm is attributed primarily to improvements in obstetric and neonatal care. Administration of antenatal steroids [123], female sex [124], surfactant treatment [125, 126], absence of fetal risks factors such as IUGR and malformations as well as neonatal low scores at birth [127] are associated with better survival rates. Centralization of perinatal health care [128, 129] and the attitude of attending obstetricians and neonatologists regarding the newborns chances of survival and the preterm delivery [130, 131] have also been shown to influence the short term prognosis.

Prenatal treatment with corticosteroids

Administration of corticosteroids to enhance maturation of the lungs of the preterm infant belongs to the most important advances in perinatal care. Such treatment at least 24 hours prior to delivery appears to attenuate or even prevent the incidence and severity of respiratory distress syndrome (RDS) as well as mortality and

intraventricular hemorrhage [132]. The consensus statement developed in 1994, concluded that antenatal corticosteroid therapy reduces mortality, respiratory distress, and the incidence of IVH in infants born between 24 and 32 weeks of gestation.[133] Following the international guidelines.[134, 135] Swedish recommendation states that 12 mg betamethason should be administrated intramuscularly twice with 24 hours apart. The treatment should be given in between gestational 23 to 34 weeks of gestation. [134]

Treatment with tocolysis

Perinatal death and morbidity are strongly related not only to low gestational age at birth, but also to whether or not antenatal corticosteroids are administered and whether the preterm infant is transferred to a tertiary care centre before or after birth. Postponement of delivery for 48 hours with tocolytics in order to allow steroids to have a maximal effect and give time for transfer of the mother to a centre with Neonatal Intensive Care Unit (NICU) is therefore standard treatment whenever there is a risk for preterm labour. In many patients tocolytics only stop contractions temporarily, so that delay of labour until term is not achieved.

Since recent meta-analyses have failed to demonstrate any improvement in neonatal outcome with use of tocolytics, and the maternal/fetal side-effects are unknown, the continued application of these drugs must be questioned. In general, if tocolytics are administrated, they should be given together with corticosteroids since it`s only than the neonatal morbidity is reduced. [132]

The GA at which tocolytics should be employed is somewhat controversial. However, since corticosteroids are generally not administered after 33 weeks and perinatal outcomes in preterm neonates are generally favorable after this age, most practitioners do not recommend administration of tocolytics at or after 34 weeks. The American College of Obstetricians and Gynecologists [136],later joined by the Swedish Society of Obstetrics and Gynecology recommends that tocolysis should be considered when there are regular uterine contractions together with documented cervical change .

The growing number of drugs utilized to delay or prevent preterm birth includes beta-adrenergic receptor agonists, magnesium sulfate, prostaglandin inhibitors, calcium channel blockers and the oxytocin antagonist - atosiban. Atosiban is recommended because of few side effects. [137, 138]

Advanced intensive neonatal care

The complex nature of intensive care for preterm infants' demands highly qualified staff with access to advanced technologies. Evaluations in several countries have revealed that mortality rates are lower in large (level III) than in small (level II) neonatal intensive care units (NICU) [90, 139-141]. The presence of a neonatologist at the clinic is also associated with reduces mortality and morbidity [141] .

Sweden is divided into seven regions, each with its own level III perinatal unit. Advanced intensive neonatal care is provided by the University hospitals i.e. level III hospitals situated in Stockholm, Gothenburg, Lund, Örebro, Umeå, Uppsala and Linkoping. Although, advances in modern neonatal intensive care have clearly

contributed to reduced neonatal morbidity and mortality, the benefits of a proactive versus a more selective attitude in the management of preterm infants remains controversial.

3.2.5 Selection bias

The significantly different reported rates of neonatal survival among extremely preterm born infants may reflect the use of different inclusion criteria. [142, 143] Access to reliable data concerning the survival and morbidity allows clinicians to reliably counsel pregnant woman with regard to the potential survival of her preterm child. Moreover, comparison of the survival rates at different institutions is usually considered to be an indicator of quality of obstetric and neonatal services.

The numerous publications documenting survival among preterm infants in relation to gestational age at birth provide widely varying values, which may reflect differences in study population, socio-demographic characteristics or the time period of the study. In addition, there is a potential for selection bias. Most investigations of neonatal mortality include either live-born infants or infants admitted to the neonatal intensive care unit [142] i.e. infants with a better prognosis which is likely to overestimate of survival.

Therefore, the presented evaluation of the rates of survival, current obstetric and neonatal praxis and morbidity among preterm infants in Sweden, include all infants born, either live or stillborn prior to 27 weeks of gestation.

4 METHODS

4.1 SETTING

The studies in this thesis were conducted in Sweden during the period between 2007-2012.The reported conclusions are based on the results from the cohort studies. Study material was obtained from national based registries: Medical Birth Registry (MBR) and Extremely Preterm Born Infants in Sweden (EXPRESS) registry.

4.2 DATA SOURCE

4.2.1 Medical Birth Registry

The purpose of the Swedish Medical Birth (MBR) registry is to compile information on antenatal and perinatal factors and their importance for the health of the infant. The basic structure of the registry, established in 1973, did not change during the years, but some major modifications to content and methods of data collection have been performed. In 1982 the content of the registry was expanded and a new revised data collection including information on estimated date of delivery according to LMP (EDD-LMP) and according to ultrasound examination (EDD-US) went into effect. In 1990, the MBR was further modified, and the record forms were changed. From the 1992 and onwards, maternal weight measured at the first visit to the antenatal care center is directly recorded.[144]

Today, the set of data containing 66 variables, such as the information about previous reproductive health, height, weight, smoking habits and drug use, medication, family situation, is collected prospectively at the women's first visit to antenatal care and is recorded by the midwife. The information about delivery hospital, length of gestation, type of delivery, diagnoses of mother and child is collected when the women are discharged from hospital. Women are identified by their unique personal identification number. Data is collected through copies of the standardized antenatal, obstetric and neonatal records which are sent to the Swedish National Board of Health and Welfare. The registries' quality has been evaluated three times: in 1976, 1988 and 2001. According to the latest evaluation, the register contains information of more than 99% of all births in Sweden.[145]

4.2.2 Extremely Preterm Born Infants in Sweden study (EXPRESS) registry

EXPRESS registry is a quality registry with the primary aim to investigate incidence, mortality and morbidity of infants born before 27 weeks of gestation. The data was collected during a 3-year period, from April 1, 2004 to March 31, 2007 and includes all live-born infants at gestational age ≤26 weeks + 6 days and stillborn infants at gestational age between 22 weeks + 0 days and 26 weeks + 6 days. Information on

maternal medical and previous obstetric history, data on pregnancy, labor, and delivery, infant condition, including condition at admission to neonatal intensive care unit, neonatal procedures, and infant outcomes and diagnosis as well as neonatal mortality and time of death were collected by local staff and transferred electronically to a central database. Totally, 220 variables were registered for each pregnancy. Termination of pregnancy and infants born outside Sweden were excluded.

Sweden is divided in 7 health regions and each region is served by a regional lever III hospital where one obstetric and one pediatric study coordinator were responsible for data collection. For validation control, an internal and an external control were performed on a randomly selected set of study patients. Records with missing data or obviously erroneous information were traced until the investigators concluded that data were unobtainable. The database was created in collaboration with the Swedish Perinatal Quality Register.

4.3 STUDY DESIGN AND SUBJECTS

4.3.1 Obesity and estimation of gestational age by ultrasound

The aim of this study was to evaluate the impact of maternal overweight on the dating of pregnancy based on measurements by ultrasound examination. The purpose was to investigate the risk for GA adjustment at mid-trimester ultrasound examination among overweight and obese mothers.

From the MBR we identified 868,451 singleton pregnancies for which the estimated date of delivery according to the LMP (EDD-LMP), and according to the US (EDD-US), as well as maternal BMI in early pregnancy was known. We included all singleton pregnancies with available records of maternal smoking habits, maternal age at delivery, gender of the newborn, date of birth and birth weight.

All pregnancies with adjustment of more than 30 days due to erroneous measurements or to oligomenorrhea were excluded, and the remaining 842,083 women were categorized according to maternal BMI. We used international definitions for overweight and obesity, and divided the study group into lean (BMI <20. 0 kg/m²), normal (BMI 20.0-24.9 kg/m²), overweight (BMI 25.0-29.9 kg/m²) and obese (BMI ≥30 kg/m²).[146] BMI groups were then subdivided into three groups depending on the difference between EDD-LMP and EDD-US; group 1 included pregnancies with EDD-LMP –EDD-US ≤- 7 days, group 2 those with EDD-LMP-EDD-US from -6 to +6 days, and group 3 included pregnancies with EDD-LMP-EDD-US ≥+7 days. Negative adjustment represented pregnancies that were shorter according to the examination by ultrasound than according to the estimation according to LMP. Group 2, which included pregnancies with a discrepancy between EDD-LMP and EDD-US less than 7 days, was considered the reference group. The probability for adjustment of the estimated date of delivery in different BMI groups was calculated. Potential confounders included in the analyses were maternal age, year of birth, parity and smoking.

4.3.2 Extreme prematurity

Paper I, III and IV are based on the study database of extremely preterm born infants in Sweden (EXPRESS). From the EXPRESS study registry, we obtained information on all live-born prior to 27 weeks of gestation and stillborn infants at GA 22 weeks to 27 weeks.

During the study period 1011 infants were born before 27 weeks of gestation from 904 deliveries to 887 mothers, 707 were live-born and 304 stillborn.
The information on GA at birth was collected from the hospital charts and in 95% of the cases estimation of GA was based on ultrasound examination. In 16 pregnancies (2%) the GA was based on LMP and in 28 (3%) pregnancies the dating method was not specified.

Live-birth and perinatal mortality were defined in accordance with WHO. [147] Perinatal deaths included stillbirths and intrapartum deaths. Live-born infants could die in the delivery room and at neonatal age: early neonatal death (0-6 days), late neonatal death (7-27) and infant death (0-365 days).

The major neonatal morbidity included severe intraventricular hemorrhage (IVH >grad 2), cystic periventricular leukomalaci (cPVL), retinopathy of prematurity> grade 2 (ROP), necrotizing enterocolitis (NEC), and severe BPD. The diagnoses were all defined according to the international standards. [99, 100, 148-150]

Intrauterine growth was evaluated in accordance with the national fetal weight-based growth standard. [151] Birth weight was expressed as mean standard deviation scores (SDS), calculated as (actual value - reference mean)/standard deviation. Infants with an actual birth weight more than 2 SD below the expected birth weight were classified as SGA and those with birth weight more than two SD above the expected value were considered large-for-gestational age.

4.3.2.1 Survival of extremely preterm infants

The aim of this study was to determine the survival in all infants born extremely preterm and to investigate in detail the perinatal factors that influence mortality in this group.

Maternal characteristics and live-born characteristics of infants born before 27 weeks of gestation were described in detail. We divided mothers according to their age into mothers younger than 20 years, age 20-35, age 35-39 and older than 40 years. Furthermore, mothers were divided according to smoking habits into smokers and non-smokers, and according to the place of birth into Nordic or non-Nordic origin. For description of the pregnancy characteristics, we used variables such as previous delivery, in vitro fertilization, and pregnancy complications, e.g., preeclampsia, antepartum hemorrhage, PPROM or chorioamnionitis.

The characteristics of live-born infants were stratified according to the GA into ≤22 weeks, 23 weeks, 24 weeks, 25 weeks, 26 weeks and total <27 weeks. The characteristics described were: gender of the infant, multiple pregnancies, Apgar score

≤3 at 1 minute and at 5 minutes, SGA, birth weight, birth weight SDS, congenital anomalies. Perinatal interventions included medical treatments prior to delivery, type and place of delivery and neonatal interventions (neonatologist attending at birth, intubation at birth, surfactant administration, admission for neonatal care and transport to level III hospital). Iatrogenic delivery was defined as delivery for maternal, fetal or both indications either after induced labor or as prelabour cesarean delivery.[80] Mothers were considered to have received antenatal corticosteroids if they have received 1 or 2 doses of betamethasone and tocolytic therapy if they have received any tocolytic drug during hospitalization. Perinatal interventions were described for the study group stratified according to the GA.

For every GA at birth (expressed in completed weeks), survival of live-born infants was described depending on the time of death. The analysis of major neonatal morbidity was performed on 497 infants who survived to 1 year and described for each gestational week at birth. The risk for neonatal death was estimated in relation to the perinatal interventions.

4.3.2.2 Dating of pregnancy by ultrasound among extremely preterm infants

In paper III, we compared the estimation of gestational age by using existing ultrasonographic dating formulae based on the measurements of fetal biparietal diameter (BPD) and femur length (FL).

The study population was derived from the EXPRESS registry and included pregnancies with valid measurements of the fetus obtained at ultrasound examination between 12+0 and 19+6 gestational weeks, EDD according to the LMP and EDD according to the US. To avoid the potential systematic error when GA is based on measurements of the larger twin [152] as it is practiced in Sweden, we considered only the measurements of the first recorded twin in the medical chart. The recorded GA at birth was considered as the "reported GA".

Totally 513 pregnancies were included. Since in Sweden, the estimation of GA is in Sweden recommended to be based on measurements of BPD and FL, we compared three ultrasonographic formulae based on biparietal diameter and femur measurements. We recalculated GA by using formulae published by Hadlock et al., Persson and Weldner, Mul et al. and by using the information on LMP. [29, 31, 32] In the further analysis, we compared the GA distribution for these methods.

4.3.2.3 Survival and neonatal morbidity depending on method for GA estimation

The major objective of this study was to investigate the impact of method for GA estimation on perinatal mortality and neonatal morbidity. We compared the estimation based on biometric measurements by ultrasound and calculation based on LMP. The study group in paper IV was selected from the EXPRESS registry. We included pregnancies with available information on gestational age according to LMP (GA-LMP) and based on ultrasound measurements (GA-US). We excluded pregnancies with obviously erroneous data (where the GA-US minus GA-LMP was more than 30 days) and the remaining 645 were included for further analysis. Neonatal mortality, rates of stillbirth, birth weight and major neonatal morbidity were defined as study outcomes.

Major neonatal morbidity included ROP, IVH, cPVL, severe BPD and NEC. The GA distribution and rates of stillbirths, neonatal survival and morbidity were then calculated according to the LMP and according to the US. The cohort was then divided into 3 groups in accordance with the discrepancy between GA-US and GA-LMP. Group 1 included pregnancies where the fetus was at least 7 days smaller than expected at ultrasound examination and thereby the due day was postponed by 7 days. The group 2 was considered as the reference group and in group 3 the discrepancy between GA-US and GA-LMP was more than + 6 days. For each group we calculated the OR for stillbirths, neonatal death, SGA and major neonatal morbidity in comparison with reference group, i.e. the pregnancies with adjustment +/- 6 days.

4.4 STATISTICAL METHODS

4.4.1 Survival of extremely preterm infants

The main study outcome measures defined as infant survival to 365 days and survival without major neonatal morbidity were calculated as incidence rate (number with event/number in group) and presented in %. The overall survival of infants born alive according to gestational age was determined by Kaplan-Meier survival analysis. Fetal risk factors for infant death (infant gender, SGA, multiple birth) were evaluated using multiple logistic regression analysis adjusted for possible confounder (gestational age).

The effect of perinatal interventions was evaluated by simple logistic regression and adjusted for gestational age. The multivariate model including gestational age and all evaluated interventions was performed in order to estimate OR for specific perinatal intervention (tocolytic treatment, cesarean delivery, administration of corticosteroids, treatment with surfactant, and birth at level III hospital).

4.4.2 Maternal obesity and estimation of gestational age by ultrasound

In order to evaluate the probability for the adjustment of GA estimation among overweight women, the multiple logistic regression analysis with BMI as class variable was performed. The adjustment of GA was defined as the difference in estimated day of delivery by LMP and US (EDD-LMP minus EDD-US). The calculation of OR with 95% confidence interval was adjusted for continuous variables (year of birth, maternal age, parity) and maternal smoking (divided into non-smokers, < 10 cigarettes per day and ≥10 cigarettes per day). Women with BMI of 20.0-24.9 kg/m^2 served as the reference group. The association between maternal BMI and discrepancy between EDD-LMP and EDD-US of ≤-7 days and ≥ 7 days compared with +6 to -6 days were investigated using a model in which maternal BMI was entered as a third-grade polynomial.

4.4.3 Ultrasonographic dating formulae among extremely preterm infants

In order to investigate distribution of GA according to three ultrasonographic formulae and LMP, the mean, ranges and the 25th, 50th and 75th percentiles for each method were calculated. The overall significance of the differences in GA distribution was analyzed by the Friedman test. Furthermore, the gestational age according to the dating formula by Hadlock, Mul, Persson and LMP were pairwise compared by Wilcoxon's sign rank test (p-values <10-6). The birth weight and incidence of SGA were calculated for each dating formula. For pair-wise comparisons of the SGA rates we used the MacNemar test.

4.4.4 Survival and neonatal morbidity depending on method for GA estimation

Gestational age of all infants with available data in the cohort was calculated according to the LMP and according to the ultrasound examination. The mean GA for each method was calculated and the correlation between two methods was estimated by Spearman rho test (95% CI). The difference between two groups was evaluated by Willcoxon's signed rank test. The neonatal survival in the group where GA was estimated by LMP and in the group where GA was estimated according to ultrasound was evaluated by Kaplan-Meier survival analysis.

The discrepancy in GA estimation between two methods was expressed in days. We compared the differences of at least 7 days to the reference group (GA-US minus GA-LMP +/- 6days) and calculated OR for stillbirth, neonatal death and morbidity using logistic regression analyzes. GA, maternal age, parity, smoking and BMI were introduced into analyzes as possible confounders.

4.5 ETHICAL CONSIDERATIONS

4.5.1 Research based on data collected from the EXPRESS registry

The study was approved by Regional Research Ethics Board, Lund University, Lund, Sweden. All patients included in the registry received written and oral information twice; once at the admission to the obstetrical clinic and once at the admission to the neonatal intensive care unit. The information was given in accordance with the recommendation of the Medical Research Council and included everything that was reasonably considered to have an effect on the subject's decision to participate. The participants could demand to be excluded from the register at any time during the study. In accordance with Swedish patient data low, the information was depersonalized and stored at the protected hard disk.[153]

4.5.2 Research based on data collected from MBR

The Swedish Medical Birth Register is mandatory register that includes all patients delivered since 1973 in Sweden. Personal data in the Medical Birth Registry may be used for the production of statistics, for monitoring and evaluating the quality of health care, for research and epidemiological studies in the reproductive health, for surveillance of birth defects as well as newborn and children health. Our study was approved by Regional Research Ethics Board, Lund University, Lund, Sweden. Since the participation in the register is compulsory and the patient cannot decline the participation in the study or data acquisition, no informed consents were provided. In accordance with the regulation provided by the Swedish National Board of Health and Welfare all data was depersonalized and stored at the safe place.[154]

5 RESULTS

5.1 SURVIVAL OF EXTREMELY PRETERM INFANTS

Mortality and survival

The primary outcome of the study was infant survival to 365 days of all extremely preterm born infants, stillbirths as well as live-born infants, included in the EXPRESS registry. Overall survival at 1 year of age among 707 live-born infants was 70 % with rates increased with advancing gestational age; at 22 weeks the survival rate was 9.8% and at 26 weeks it was 85%. In the analysis adjusted for GA, both SGA (OR 1.69; 95 % CI 1.12-2.58) and multiple birth (OR 1.70; 95% CI 1.04-2.77) were associated with increased risk for infant death. The overall mortality in cohort of extremely preterm born infants was 45 % and the rates were related to GA, increasing with decreasing GA.

Maternal and live-born infants' characteristics

During the study period (2004-2007), 1011 infants were born before 27 weeks of gestation. Totally, 887 mothers were included and 904 deliveries registered.
The oldest mother was 46 years and youngest 14 years old (mean age 30.9 years). Most of the mothers were primiparae (58%) and from the Nordic countries (80%). Totally 102 multiple births (11.3%) were registered and 6.6% of pregnancies were the result of in vitro fertilization.

Among infants in the study, the incidence of stillborn were 1.0 /1000 infants and of live-born 2.3/1000.

The rates of SGA as well as the total number of infants increased with gestational age; at 23 weeks of gestation 7 % (7/100) were diagnosed as SGA, at 26 weeks of gestation 23% (48/206) infants were SGA.

Survival without major neonatal morbidity

Totally, 226 (45%) infants of those who were born alive survived 1 year without major neonatal morbidity. The percentage increased statistically significant with GA and ranged from 20% at GA week 22 to 63% at 26 weeks. In the study population, 10 % developed severe IVH, 34% ROP, 25% was treated for severe BPD, 5.6% and 5.8% for PVL and NEC, respectively. (Table 1)

Table 1. One-Year Survival, Major Neonatal Morbidity Among Survivors, and Survival Without Major Neonatal Morbidity

	No. With Event/No. in Group (%)					
	Gestational Age, wk					
	≤22[a]	23	24	25	26[b]	Total <27 wk
Survival 365 days						
All infants including stillbirths	5/142 (3.5)	53/183 (29)	96/191 (50)	167/250 (67)	176/245 (72)	497/1011 (49)
Live-born infants	5/51 (9.8)	53/101 (53)	96/144 (67)	167/205 (82)	176/206 (85)	497/707 (70)
Infants admitted to neonatal intensive care unit	5/19 (26)	53/81 (65)	96/132 (73)	167/200 (84)	176/204 (86)	497/636 (78)
Survival 365 days with major neonatal morbidity[c]						
Intraventricular hemorrhage grade >2	1/5 (20)	10/53 (19)	10/96 (10)	20/166 (12)	9/173 (5.2)	50/493 (10)
Retinopathy of prematurity stage >2	4/5 (80)	33/53 (62)	45/94 (48)	54/167 (32)	33/174 (19)	169/493 (34)
Severe bronchopulmonary dysplasia	2/5 (40)	13/49 (26)	27/87 (31)	45/153 (29)	26/158 (17)	113/452 (25)
Periventricular leukomalacia	0/5 (0)	5/53 (9.4)	6/96 (6.2)	9/167 (5.4)	8/176 (4.5)	28/497 (5.6)
Necrotizing enterocolitis	0/5 (0)	1/53 (1.9)	9/96 (9.4)	10/167 (6.0)	9/176 (5.1)	29/497 (5.8)
Survival 365 days without major neonatal morbidity[d]						
Live-born infants	1/51 (2)	9/101 (8.9)	30/144 (21)	75/205 (37)	111/206 (54)	226/707 (32)
Survivors at 1 year	1/5 (20)	9/53 (17)	30/96 (31)	75/167 (45)	111/176 (63)	226/497 (45)

[a] Category denotes gestational age of 22 weeks 0 days to 22 weeks 6 days but also includes 1 infant born at 21 weeks 5 days and 1 infant born at 21 weeks 6 days.
[b] Category denotes gestational age of 26 weeks 0 days to 26 weeks 6 days.
[c] Infants with known information in denominator.
[d] Survival without any of the major neonatal morbidities (intraventricular hemorrhage, retinopathy of prematurity, severe bronchopulmonary dysplasia, periventricular leukomalacia, or necrotizing enterocolitis) as described. Denominators are for all infants, including 21 infants without reported morbidity but with some information missing.

Perinatal interventions and neonatal survival

Most infants in the study were born at level III hospital (70%) and neonatologist attended 83% of live-births. Perinatal interventions depended on the GA and the treatment with tocolytics, antenatal corticosteroids and surfactant were used significantly less at 22 weeks than at later GA.

The chance of survival was increased by antenatal treatment with tocolytics (OR 0.43; 95% CI 0.36-0.52), corticosteroids or both (OR 0.44; 95% CI 0.24-0.81), surfactant treatment at 2 hours after birth (OR 0.47; 95% CI 0.33-0.68) and birth at level III hospital (OR 0.49; 95% CI 032-0.75) while delivery by cesarean section did not influence the OR for neonatal survival. The OR remained significantly increased even when a multivariate model was introduced except for birth at level III hospital (OR 0.78; 95% CI 0.45-1.35).

5.2 MATERNAL OBESITY AND ESTIMATION OF GESTATIONAL AGE BY ULTRASOUND

In the group of overweight and obese mothers, 25 % and 31.9%, respectively, had discrepancy between estimated day of delivery based on LMP (EDD-LMP) and on ultrasound examination (EDD-US) of at least 7 days, while corresponding prevalence for normal-weight mothers was 23, 7 %.

The risk for the overweight and obese mothers to be postponed at ultrasound examination was evaluated in the multivariate logistic regression analysis adjusted for year of birth, maternal age, parity and smoking. The obese mothers were significantly more likely to have the EDD postponed i.e. fetuses were smaller at ultrasound examination than according to the LMP, compared with the reference group. Mothers with BMI ≥ 30 had increased risk for postponing of EDD between 7 and 13 days (OR, 1.45; 95% CI, 1.42–1.48) and even significantly elevated risk for postponing of at least 14 days (OR 1.65; 95% CI 1.60-1.70) in comparison with women with normal weight.

Obese women were also slightly more likely to have discrepancy between EDD-LMP and EDD-US more than +14 days compared to the reference group but the magnitude of this association was weaker than was the association between obesity and postponement. (OR 1.28; 95% CI 1.20-1.37)

As shown in Figure 1, the OR for discrepancy obtained by the third-grade models agreed well with the results from the models using BMI as class variables. The results suggest a continuously increasing risk for discrepancy of ≤ - 7 days with increasing maternal BMI, while the risk for ≥ + 7 of days increase was comparatively in lower magnitude. This association did not change over the study period (P for homogeneity = 0.53)

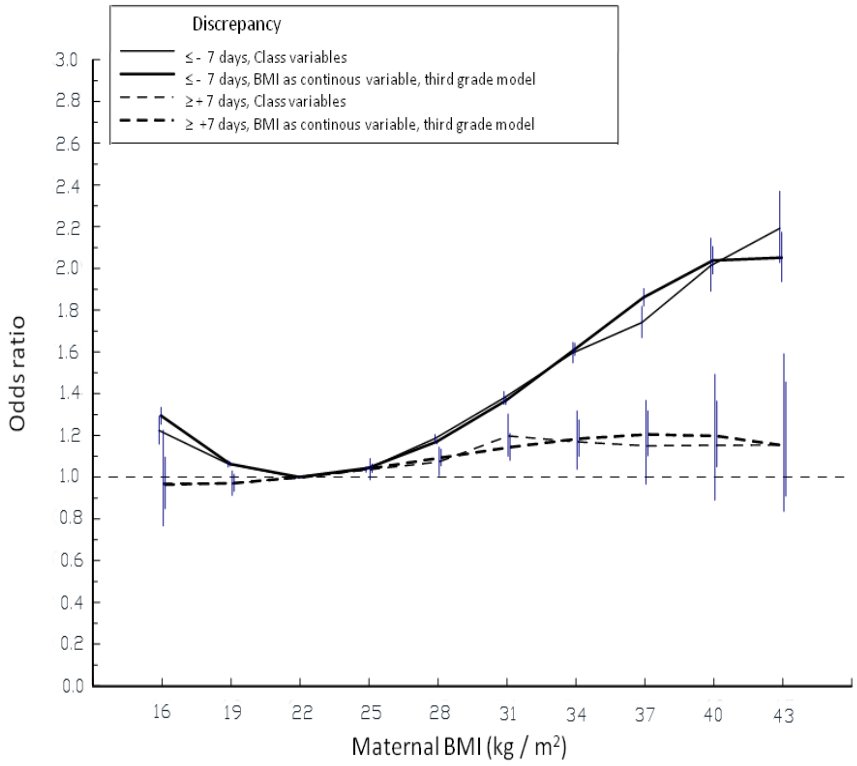

Fig 1.The association between maternal body mass index (BMI) and discrepancy between the estimated date of delivery according to the LMP and according to the ultrasound

During the study period, 842,083 women had available information on maternal BMI, EDD-LMP and EDD-US. In the study population, 56.9% of the mothers had a normal weight while 31, 8 % were overweight (23.0% had BMI 25-29.9, 6.5 % had BMI 30.0-34.9, 1.6% had BMI 35.0-39.9 and 0.7 % had BMI >40 kg/m^2) Obese mothers were more likely to be older, multiparous and smokers but also more likely to deliver port-term LGA infants. During the study period 53,476 infants were born prior to 28 weeks of gestation (incidence 0.2%).

5.3 ULTRASONOGRAPHIC DATING FORMULAE AMONG EXTREMELY PRETERM INFANS

In this study we compared duration of gestational age (GA) as reported in the EXPRESS registry, with GA estimates based on LMP and on ultrasound examination by using three ultrasonographic dating formulae. The results of the study were based on the 513 pregnancies with valid information on fetal biparietal diameter and femur length and LMP records.

The mean reported GA (173.2 days) in the EXPRESS study corresponded well to the mean GA when calculated according to the Persson & Weldner dating formula (173.3 days). The GA according to the LMP, formulae published by Hadlock and by Mul differed significantly and resulted in on average longer pregnancy duration than the reported GA. When we stratified the material for GA at birth, we observed that if GA was calculated according to the LMP, 16% of pregnancies were older than 27 weeks which was the inclusion criteria for the study. The corresponding percentages based on the dating formulae by Hadlock et al., Mul et al., and Persson & Weldner were 10%, 6%, and 2%, respectively. (Figure 2) The GA estimates by three ultrasonographic dating formulae differed significantly between each other (p<10-6 Wilcoxon´s sign rank test). Furthermore, in the EXPRESS registry, 68 pregnancies had reported duration of 22 weeks. Among these pregnancies 22 (32%) and 33 (49%) had duration of 23 weeks or more if GA was calculated according to the formula by Hadlock and based on LMP, respectively.

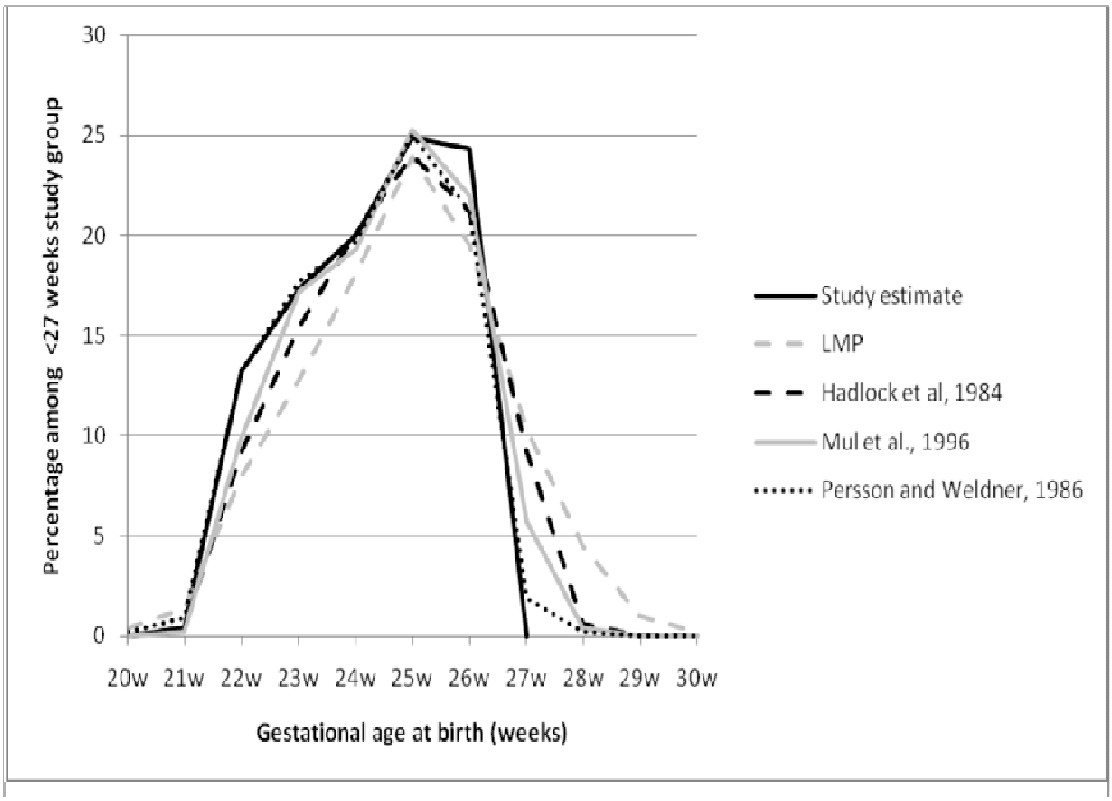

Fig. 2. Distribution of the gestational age according to the applied formulae.

The rates of live-born infants classified as SGA ranged from 31% for GA according to LMP to 23%, 22% and 19% for GA according to Hadlock et al, Mul et al and Persson & Weldner formula, respectively. In 73 out of 353 live-born infants (21.0%) the GA obtained by using Persson & Weldner was shorter at least seven days than according to the LMP. This group had increased risk for SGA (OR 2.02; 95% CI 1.04-3.82) comparing to pregnancies without such a discrepancy. When Hadlock et al formula was applied, the risk for SGA was elevated but with less significance (OR 1.91; 95% CI 0.90-3.91).

5.4 SURVIVAL AND NEONATAL MORBIDITY DEPENDING ON METHOD FOR GA ESTIMATION

The results in this study show significant difference in distribution of gestational age depending on the method used for estimation of gestational age.

The mean gestational age based on ultrasound (GA-US) was 24.7 weeks (95% CI: 24.6-24.8), whereas the mean gestational age according to LMP (GA-LMP) was 25.3 (95% CI: 25.2-25.4). Out of 645 infants born before 27 weeks of gestation, 111 (17.2 %) infants had at birth higher GA than 27 weeks when GA was calculated by LMP. Overall, the infants tended to be older when GA was based on LMP.

	Gestational age according to ultrasound (weeks)						
	<22 (N=2) n (%)	22 (N=76) n (%)	23 (N=120) n (%)	24 (N=129) n (%)	25 (N=164) n (%)	26 (N=154) n (%)	Total <27 (N=64) n (%)
GA based on LMP							
<22	1 (50.0)	6 (7.9)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	8 (1.2)
22	1 (50.0)	31 (40.8)	8 (6.7)	3 (2.3)	1 (0.6)	2 (1.3)	46 (7.1)
23	0 (0.0)	23 (30.3)	47 (39.2)	6 (4.7)	2 (1.2)	3 (1.9)	81 (12.6)
24	0 (0.0)	11 (14.5)	37 (30.8)	57 (44.2)	11 (6.7)	6 (3.9)	122 (18.9)
25	0 (0.0)	2 (2.6)	18 (15.0)	43 (33.3)	74 (45.1)	5 (3.2)	142 (22.0)
26	0 (0.0)	3 (3.9)	5 (4.2)	11 (8.5)	53 (32.3)	63 (40.9)	135 (20.9)
≥27	0 (0.0)	0 (0.0)	4 (3.3)	9 (7.0)	23 (14.0)	75 (48.7)	111 (17.2)

Table 2. Relationship between the gestational age (GA) of infants born extremely preterm based on the last menstrual period (LMP) or estimated by ultrasound examination.

In 28 pregnancies the fetus was larger at ultrasound examination than expected by LMP (GA-US minus GA-LMP > 7 day). We excluded 14 pregnancies due to probably erroneous LMP data (birth weight was more than 2 SD above the expected weight on the basis of GA-LMP). Among infants that were larger than expected at ultrasound examination, five demonstrated severe IVH/cPVL and this risk remained elevated even after adjustment for possible confounders (data not shown).

As shown at Figure 3, the survival rates for infants born between 22 and 26 weeks of gestation were similar for both methods. However, pregnancies that were longer than 27 weeks according to the estimation by LMP, had lower survival rate than infants born at a GA-US of 25 and 26 weeks.

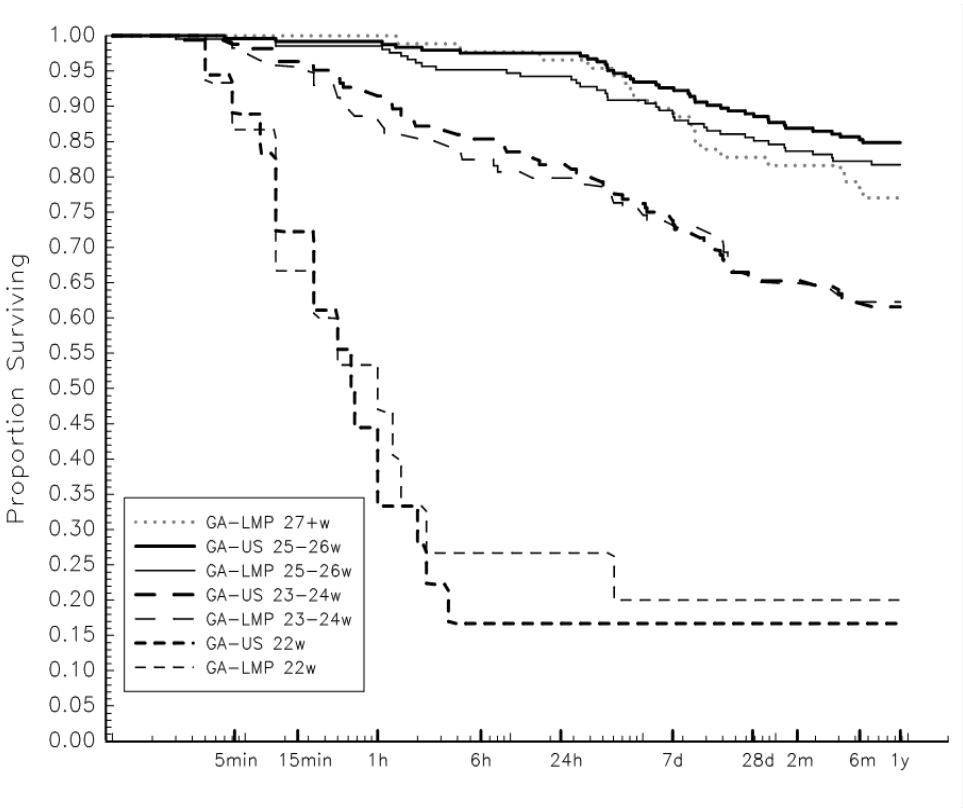

Figure 3: Survival among infants born extremely preterm according to the gestational age at birth and method of gestational age estimation

The incidences of stillbirth demonstrated a similar distribution in the case of both GA-LMP and GA-US. Stillbirth was most frequent (76.3 %) among infants born at a GA-US of 22 weeks and least frequent (20.1 %) at 25 weeks of GA-US, with the corresponding incidences at the GA-LMP being 58.7% and 21.8%, respectively.

Further, we compared the risk for adverse neonatal outcome among fetuses that were smaller at least 7 days than expected at ultrasound examination (GA-US minus GA-LMP < -7 days) with the reference group i.e. fetuses that were of expected size at ultrasound examination (GA-US minus GA-LMP +/- 6 days). In the group of infants that were smaller than expected, the risk for SGA and stillbirth were elevated but there were no differences with respect to major neonatal morbidity.

6 GENERAL DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

Epidemiological research is, to a large extent observational rather than experimental, with the disadvantage that extraneous factors cannot be controlled by the investigator. Cohort and case-control methodologies are major approaches employed in analytical epidemiological research, and the cohort studies described in the present thesis are based on material collected from the registries data.

The cohort study is designed to examine the influence of a certain exposure on a given outcome as well as the occurrence of the disease, by comparing two groups of individuals, one that received the exposures of interest and one that did not, and following these cohorts forward for the outcome of interest. This observational approach can be prospective or retrospective.[155] The latter is a more efficient way of collecting large amounts of data over a long period of time, but is limited to the information on exposure and potential confounders included in the registry employed. Here, the risk factor for miscalculation (adjustment) of gestational age among obese pregnant women was determined from data collected retrospectively from the Medical Birth Registry (MBR).

Characterization of the infants born extremely preterm was based on the EXPRESS registry, with data being collected prospectively in this case. This is more time consuming and therefore more expensive but the information concerning exposure, confounders and outcome could be better defined to suit the purposes of the study aim.

6.1.1 Internal validity

The internal validity of a study reflects the accuracy of its conclusions about the effects of an intervention on a given group of subjects under specific circumstances. Lack of internal validity may arise from either random and/or systematic error.

6.1.1.1 Random error

A random error in a cohort study consists of variability, positive or negative, due to chance and it reflects the precision. Statistical procedures can be applied to assess the role of chance which is described by confidence interval and p-value. The confidence interval employed here was 95%.

6.1.1.2 Systematic error

A systematic error or bias occurs when there is a difference between the true and observed value due to any cause other than sampling variability and it represents a problem of validity. Biases are commonly grouped into three general categories, i.e., selection bias, information bias and confounding.[156, 157]

Selection bias

Selection bias in a cohort study may arise if the risk of experiencing the outcome of interest influences the probability of an individual being included in the cohort or if it influences the manner in which exposure is defined. Such bias may, however, also be viewed as a matter of external rather than internal validity.

The criteria for inclusion of patients in the EXPRESS registry was the preterm birth prior to 27 weeks of gestation. In 95 % of these pregnancies gestational age was estimated on the basis of ultrasound and it is well known that this procedure can involve systematic error. [152] As seen in the Paper III, 81 of the pregnancies recorded in the EXPRESS registry as less than 27 weeks appeared to be longer when gestational age was based on LMP. This might have led to the selection bias, since these infants might in reality have been small for their gestational age and perhaps growth restricted, but were included in the study as being born extremely preterm.

In attempt to avoid selection bias in examining the survival of infants admitted to the neonatal unit or live-births only, stillbirths were also included in the EXPRESS registry. However, at a low gestational age, stillbirths outside the maternity unit might have been treated as miscarriages and thereby missed which would result in underestimation of the rate of still-births among infants born extremely preterm.

Confounding

Confounding is a central issue in epidemiological studies, could simply be called confusion of the effect. A confounding factor is associated with exposure but also with the outcome but not as an intermediate step in the causal pathway between exposure and outcome. [157] If a confounder is not identified or adjusted for, the actual association between the risk factor of interest and the outcome could be distorted.

Adjustment of confounding aims to isolate the effect of a given risk factor on outcome and can be performed prior to or after an investigation. Thus, if confounders are not restricted in the study design itself, they may be adjusted for in the subsequent data analysis, provided information on the confounding factors is available. The two main approaches to controlling for confounders in connection with the analysis are stratification and regression modeling. In the cases of multiple pregnancies, most Swedish ultrasound units routinely use the measurements of the largest twin to estimate the gestational age. However, since the actual growth curve for twins is similar to that of singletons during the first trimester, [158, 159] this approach can give rise to systematic overestimation of GA in such case.[152] In attempt to avoid such bias, we included only singleton pregnancies in Papers II and IV. In the study on the comparison of formula for pregnancy dating, we utilized the measurements of the fetus who was screened first of the two twins. By this procedure we randomly collected the fetal measurements. The other method could be to use the mean of the measurements of each twin pair.

Papers I, III and IV were based on data reported in the EXPRESS registry which includes all patients who delivered prior to 27 weeks of gestation, regardless of any possible confounders. Since gestational age, demonstrates strong correlation to neonatal survival and morbidity in studies on prematurity, it was defined as a risk factor

or as a confounder, depending on the study analysis. In the analysis of survival and neonatal morbidity, the gestational age was analyzed as a risk factor.
In our investigation on the relation between perinatal interventions and neonatal outcome (paper I), the potential effect of gestational age as a possible cofounder was evaluated by multiple logistic regression analysis. Since some infants were subjected to several perinatal interventions, the multivariate model was applied to evaluate the influence of the one specific intervention treating gestational age and all other interventions as potential cofounders.

The effect of maternal obesity on the risk for discrepancy in gestational age upon ultrasound examination (paper II) was examined during a long period (15 years) with a large study derived from the MBR. Routines and guidelines for ultrasonographic examination may have changed during this long period. Moreover, several maternal characteristics, including maternal age [160, 161], parity [162] and smoking [163, 164] are associated with any increased risk for poor pregnancy outcomes. Therefore, we calculated the OR for discrepancy in estimation of gestational age using multiple logistic regression analysis with maternal smoking, parity, maternal age and year of delivery as covariates.

Information bias

Information bias, also referred to as observation bias, results from incorrect collection of information concerning exposure or/and outcome and can introduce systematic error. Non-differential misclassification results from such inaccuracies of similar magnitude with respect to both the exposed and unexposed cohorts.

A major source of misclassification in connection with reproductive epidemiology concerns ascertainment of gestational age. In Sweden, gestational age is estimated on the basis of ultrasound examination during the early second trimester ultrasound if available (95% of all pregnancies) and otherwise on the basis of last menstrual period. In the case of Paper II, the period examined was 1992-2007, during which all women in Sweden were offered routine ultrasound screening, with approximately 95% being scanned.[165] Thus, most durations of pregnancy registered in the MBR are also based on ultrasound measurement, as are 95% of those reported in the EXPRESS registry. Since the method for ascertainment of gestational age was distributed similarly in the cohort, this represents, if anything, a non-differential misclassification of minor concern.

Perinatal intervention and neonatal outcome were defined precisely prior to the start of the EXPRESS study and the individuals who registered this information were well informed and strongly motivated. Nonetheless, it should be kept in mind that certain pregnancies and infants may have been misclassified, thereby introducing an error. Moreover, the misclassification of exposure should be considered when the information is self-reported. Information on smoking habits during pregnancy was obtained at the time for registration to antenatal care (usually during gestational weeks 8-12), and we do not know how many responded accurately or who continued to smoke during pregnancy. Thereby, there is a risk for underestimation of such exposure. The maternal weight was collected prospectively without knowledge about outcome. Furthermore, it was measured by midwives and not self-reported which further minimized the risk for the recall bias.

A potential source of recall bias involves information concerning the last menstrual period. In Paper II, we assumed that mothers of normal weight exhibited the same recall bias as those who are overweight and thus, that any misclassification was of non-differential character.

Neonatal outcome in the assessment of studies on extreme prematurity was defined strictly in accordance with international recommendations and current clinical guidelines. However, the diagnosis of SGA was based on curves for normal birth weight versus gestational age. Thus, if the estimation of gestational age was erroneous, the diagnosis of SGA would be incorrect, leading to misclassification of outcome.

6.1.2 External validity

External validity is a reflection of generalizability of the findings, i.e. their applicability to other, similar populations. Obviously, our observations on neonatal mortality and morbidity are applicable only to populations of extremely preterm born infants. Moreover, the relevant data were collected primary from specialized tertiary centers throughout the whole country and thus represent advanced perinatal care. At the same time, tertiary centers, which are often the source of preterm cohorts in the literature, may also treat a larger proportion of higher risk pregnancies thereby introducing selection bias, the influence of which is difficult to predict. However, the demographic data, maternal characteristics, and obstetric and neonatal routines are representative for the Swedish population during the period examined rendering our findings comparable to similar reports from other countries.

The conclusions concerning the estimation and calculation of gestational age by different methods and formula, based on the EXPRESS cohort (Paper III and IV) can be generalized to the population of extremely preterm infants, since these results are based on complicated pregnancies that would not continue until term. Our investigation of the effect of maternal obesity on the risk for discrepancy at ultrasound examination was based on the MBR which includes virtually all deliveries in Sweden. This study was based on singleton pregnancies and should be applied with caution to all pregnancies. These results are generally applicable to the population of overweight and obese mothers in Western countries.

6.1.3 Registry-based research

Sweden offers exceptional opportunities for epidemiological studies, thanks to the well organized health system, nationwide registries, and systematic use of national registration numbers. [166] [167]

The quality of medical registries is a central factor in connection with reliability, so the proportion of missing records and variables registered are concerned. Evaluation has revealed that only relatively small proportion of all births are not included in The Swedish Medical Birth Register (MBR).[145] From here, we obtained the date of the last menstrual period and the corrected estimated day of delivery based on ultrasound examination. Furthermore, maternal age (calculated from her date of birth), parity, date of admission to the hospital, time of day for delivery, infant gender and birth weight are all recorded with a high degree of validity in MBR. Information concerning smoking is lacking for no more than 4.9% and pre-pregnancy weight (in effect, weight at the time

first visit to antenatal care) is available for 70% of the women. Obviously missing data influence any estimate of prevalence, but will usually have little impact on estimates of risk (e.g. for pregnancy adjustment).

The EXPRESS registry is aimed to provide data for research and quality assessment of perinatal care, and it was constructed to suit the need of the researchers. During our study period, one internal and one external control of randomly selected subsets of data in this registry were performed. In addition, to further ensure accuracy, the information on the mothers and infants was cross-checked with the national Medical Birth Registry and the information on infant deaths with the national Population registry. Obviously erroneous or missing data were tracked down until found or until the investigators were sure that they were unobtainable. For the first time, even information on stillborn infants born prior to 27 weeks of gestation were collected and included in the registry, making EXPRESS data base representative for the study population.

6.2 FINDINGS AND IMPLICATIONS

6.2.1 One-year survival of extremely preterm infants

The most important finding in this study is the high survival rate of the extremely preterm infants born alive in Sweden. Compared to international reports, the proportion of infants with known risk factors for adverse outcome such as male sex, multiple pregnancies, and 5-minute Apgar score of 3 and less, included in the study was similar.[69, 94, 98] However, survival rates at the time of hospital discharge were higher than in other recent population based studies. [72, 94, 97] Possible explanations for the comparatively high survival rates could be fewer intrapartum deaths and high quality of neonatal intensive care in Sweden. High survival rates reflect a more proactive approach to perinatal and neonatal care including tocolytic treatment, administration of antenatal corticosteroids, intubation upon delivery and treatment with surfactant. Perinatal interventions were more selective with births at 22 weeks of gestation than at higher gestational ages which may partially explain the differences in survival rates between gestational ages. In accordance with the current recommendation, 70% of infants were born at level III hospital. However, the birth at level III hospital per se did not increase the chance for survival to one year.

The survival rates, mortality rates, major neonatal morbidity and SGA rates were all strong related to gestational age at birth. Clearly, correct estimation of GA is essential to making clinical decisions, management and prognosis of infants born extremely preterm, as well as for epidemiological and public health assessments and comparisons between scientific reports. Here, gestational age was estimated on the basis of ultrasound examination in the majority of cases (95%) in keeping with the current routine in Sweden. However, some infants with early intrauterine growth restriction may have been considered to have a falsely low gestational age. The rate of SGA increased with gestational age, which could also be a consequence of such erroneous correction of gestational age by ultrasound only a few weeks prior to birth. Possibly, at a higher gestational age intrauterine growth restriction has been present for a longer time, thus producing more fetuses who are SGA.

The differences between the many reports of highly heterogeneous cohorts of preterm infants may reflect varying socio-demographic characteristics, time periods of the study, or interventions that improve the outcome. Decisions concerning withdrawal of neonatal intensive care are known to vary between countries and even between regions [168] and might also partially explain variations in survival rates. To achieve representative findings and determine temporal trends, we conducted a 3-year study on the entire Swedish population.

When examining preterm cohorts, consisting exclusively of admissions to neonatal units or live births, selection bias can be introduced and the apparent neonatal outcome improved. Admissions to neonatal units may ignore live births that were not resuscitated and thereby overestimate the survival. Examination of live births, but not stillbirths' data may overlook pregnancies that were not monitored during labour because stillbirth was expected on the basis of poor prognostic factors. Such selection of infants and fetuses with a better prognosis is likely to lead to an overestimation of survival. [169] To avoid such bias and obtain a more representative material, all infants born alive as well as stillbirths were included in the EXPRESS study.

Even though our present findings reveal a high rate of survival among infants born extremely preterm, the prognosis based on individual assessment, including early and subsequent morbidities and parental desires, remains the most important aspects of decision making. [170]

6.2.2 Maternal obesity and estimation of gestational age by ultrasound

In this large, population-based study, we found that in comparison to mothers of normal weight, obese mothers had an elevated risk of the gestational age of their fetus being adjusted in connection with ultrasound dating at mid-trimester. The risk that the fetus was smaller than expected increased with the degree of maternal obesity.

These findings have biological, methodological and technical explanations.

Biologically, obese and overweight women experience more abnormalities of menstrual cycle, anovualtion and infertility than do their normal-weight counterparts, [171, 172] which could partially explain our observation. At the same time, this risk increased linearly with BMI and, to our knowledge, there is no report of such linear relationship between BMI and menstrual cycle length, although suboptimal visualization of fetal anatomy has been reported to increase linearly with degree of obesity.[42, 173] Postponing of the predicted delivery to a later date on the basis of ultrasound examination might reflect early intrauterine growth restriction. [54, 57] The association between maternal obesity and increased birth weight is well known, so that growth restriction is not likely to be a reason for the postponing the date of delivery. [174]

Since the information on LMP was self-reported, recall bias must be considered. However, since there is no reason to believe that obese women experience more difficulty remembering the date of their LMP than mothers with normal weight this bias is non-differential in character and does not influence the relation between exposed (obese) and unexposed (normal weight) mothers.

Our observation that the fetuses of obese women are more often smaller than expected at ultrasound examination could also be explained by technological factors. The BMI reflects overall fatness and does not take into account the distribution of body fat. Still, there is an association between BMI and abdominal fat since the depth of the external oblique muscle beneath the skin surface was significantly greater (1.8 cm) in obese individuals than in individuals with normal weight (0.95 cm).[175]

The major limiting factor in ultrasound scanning of an obese individual is the depth to which the beam must penetrate in order to reach the pregnant uterus. Adipose tissue is often echogenic, strongly attenuating the sound beam. [176] Since the distance that the beam must travel is inversely proportional to the quality of the image obtained, such absorption and dispersion worsens the quality of the image of the fetus which becomes fuzzy, riddled with noise and backscatter, and subject to artifacts. [176] It is unclear how exactly this lowered quality can disturb the measurement of BPD and FL besides the fact that the image is of less quality than among mothers of normal weight.

In attempt to overcome this problem in obese mothers, technical features such as lower emission frequencies, harmonic and compound imaging and speckle reduction filters

have been employed. [176] However, Hendler and colleague concluded that despite significant technological advances in the quality of ultrasound machinery, maternal obesity and maternal abdominal fat still exert an adverse influence on perinatal diagnosis. However, they did find that advanced ultrasound technology is somewhat beneficial for obese pregnant women examined after 18 weeks of gestation. [42] There is also evidence that a transvaginal scan at 12-15 weeks of gestation reduces the problem of the acoustic window and is the best way to visualize fetal extremities in obese mothers. [176] At 18 weeks of gestation may not be the most optimal time point for ultrasound examination for obese women who could benefit from transvaginal examination in late first trimester or if necessary a new ultrasound examination later.

During the period of examination, 53,476 infants were born prior to 28 weeks of gestation. Even though the importance of correct gestational age estimation for extremely preterm born infants is well known, the influence of maternal overweight on such estimation by ultrasonography has received only limited attention. The impact of maternal overweight on anatomical scan has been addressed by several authors. Catanzarite et al found that the overall visualization of the fetal anatomy by second trimester ultrasound deteriorated significantly with increasing maternal weight second trimester ultrasound.[177] Moreover, Wolfe et al reported marked impairment in the visualization of fetal anatomy at the threshold BMI of 36.2 kg/m2. [41] In contrast, Field and colleagues found that the maternal obesity did not decrease the accuracy of sonographic weight estimation. [178] However, findings by Field et al. illustrate the importance of fetal size in connection with the examination of obese women, since they are based on examinations performed at 25-43 weeks of gestation when the fetus is larger than at 18 weeks, the routine time for ultrasound examination.

To our knowledge, we demonstrate here for the first time that the precision of ultrasound pregnancy dating among obese women is lower than among normal weight women and, furthermore, that the findings for the former are systematically skewed.

6.2.3 Ultrasonographic dating formulae among extremely preterm infants

In the study, we found significant variations in duration of pregnancy when different dating formulae were utilized to calculate gestational age on the basis of ultrasound examination in mid-trimester. Current recommendations in Sweden state that estimation of gestational age should be based on inserting measurement of BPD and FL into suitable formula. For this reason, we compared the results of formalae derived by Hadlock , Mul and Persson and their coworkers. The gestational age calculated according to Person was closest to the gestational age reported in the EXPRESS study database since this formula is the most used in Sweden. With the Hadlock formula the pregnancies had an apparently longer average duration than that based on the reported GA.

The application of various formulae introduces systematic differences, which are usually of limited clinical significance in dating term pregnancies [179] but can influence the diagnosis and treatment of obstetric complications such as preterm birth and IUGR. In our study, 32 % of the fetuses recorded as being 22 weeks of age were older according to the formula of Hadlock. In many peri-natological centers, strict GA limits are employed for initiation of treatment of infants born extremely preterm both before and after birth. Theoretically, if the Hadlock formula had been utilized, 32% of the 22-

week old infants included in EXPRESS might have been treated differently, which would probably have had consequences for both the prognosis and outcome. However, it is at present impossible to estimate the real impact of such variations on outcome since there is no “gold standard” for estimation of gestational age.

Most countries now recommend routine ultrasound dating of pregnancy, but the particular formula chosen for routine use in many settings is often decided on the basis of consensus, rather than on evidence of accuracy or reproducibility. Moreover, even in large epidemiological studies on extreme prematurity, the method for estimating GA is not described in detail and systematic error may be present.[71, 72] In our study, 10 % of infants were older than inclusion criteria of 27 gestational weeks when gestational age was based on the dating formula by Hadlock, illustrating the impact of the choice of dating formula on study outcome. Such heterogeneity renders comparison of the rates of preterm birth and neonatal outcome between perinatal centers and populations both difficult and unreliable.

The predominant method for dating pregnancy in mid-trimester involves fetal biometric measurements by ultrasound, in particular of the biparietal diameter (BPD) [180] and/or femur length (FL) [24, 181]. By comparing these values to standardized age-for-size charts, most likely fetal age at the time of the ultrasound examination is determined. Such charts, and similar tables [28], have traditionally been based on relatively small reference studies, in which pregnant women have been subjected to an ultrasound scan at a specified time during pregnancy. The resulting measurements are usually processed by standard polynomial regression analyses to develop a model for prediction i.e. a dating formula. This traditional approach to constructing reference charts possesses a number of weaknesses. The LMP dates of the reference group of women must be known as precisely as possible, e.g. by only examining IVF pregnancies. Furthermore, because of limited resources the study populations are usually limited to approximately 500 pregnancies and involve only a few ultrasound operators.

Even the external validity of the formulae that we compared is questionable since they are based on a small number of pregnancies and were performed at least 15 years ago. For instance, Persson & Weldner based their longitudinal study on 14 pregnancies with a known date of ovulation,[31] Hadlock et al. evaluated 361 normal pregnancies in a middle-class Caucasian population between 14 and 42 weeks of gestation[29] and Muls formula published in 1996 is based on 64 pregnancies conceived with the help of assisted reproduction technique. [129] Ultrasonographic formulae used to estimate fetal weight also vary considerably in their ability to predict birth weight [182] but formulae used for estimation of gestational age, to our knowledge, have not be compared previously.

Since available dating formulae do not take into account maternal demographics, pregnancy-specific factors [183] or biological variations, customizing a formula for a particular population could be a valuable alternative.

6.2.4 Survival and neonatal morbidity in relation to the procedure for estimation of gestational age

Recently reported survival rates and neonatal morbidity among infants born extremely preterm are based on gestational age as estimated by ultrasound examination. (Paper I) In this investigation, we recalculated gestational age on the basis of the LMP and examined neonatal outcome in relation to this value. In general, the gestational age according to the LMP appeared to be longer, but, importantly, there were no differences in survival or neonatal morbidity.

Previous studies also found that LMP resulted in older estimates of GA than those derived from ultrasound examination in the mid-trimester. [47-49, 125, 184] However, the impact of this method on neonatal outcome was unknown prior to our study. In the EXPRESS study gestational age was based on ultrasound in 95% of the pregnancies; 17% of the pregnancies were longer than 27 weeks when gestational age was based on LMP. Since, as mentioned above, in the reports on several large European studies the method for estimating gestational age was not described in detail and moreover, the results for the entire cohort are combined regardless of the method used at estimation,[69, 71, 72] detailed comparisons are difficult to make. In some unexplained manner some investigators have recalculated the gestational age on the basis of the LMP, but only in order to be able to exclude pregnancies with an uncertain gestational age, i.e., those with discrepancy of more than 14 days between the two approaches. [70] Clearly, it is of very important to report the method employed to estimate gestational age estimation in studies on preterm infants.

The mean gestational age estimated on the basis of LMP was higher than mean gestational age by ultrasound i.e. the fetuses were usually smaller at ultrasound examination than expected by LMP. However, in 28 cases the fetuses were more than 7 days larger. Of these, 14 were excluded because the LMP information was probably erroneous. The risk for IVH among these infants was elevated even when adjusted for GA, maternal age, parity, smoking and BMI. The relationship between fetuses with a larger BPD than expected and macrosomia is well established, but, to our knowledge, there is no evidence for any relationship between enlarged BPD and IVH during the neonatal period. [185]

The incidences of neonatal mortality and morbidity decline with increasing GA and one should expect that, since estimation by LMP provides an older GA, the neoanatal outcome in relation to this procedure should be improved. However, the incidence of stillbirth, early neonatal death, SGA and major neonatal morbidity in the infants born extremely preterm were similar in relation to each of these two approaches for dating. To understand this, the unreliability of estimation of gestational age based on LMP must be taken into consideration.[44, 186, 187] Even more, the fetuses that were smaller than expected upon ultrasound examination ran an enhanced risk of developing IUGR and there by a higher risk for an adverse neonatal outcome.[188, 189]

In conclusion, the gestational age differs depending on the procedure employed for pregnancy dating, but despite these differences, the incidence of neonatal mortality and morbidity in relationship to both methods are similar. Our findings thus allow comparisons between various reports on outcome of preterm births in various populations.

6.2.5 Estimation of gestational age and SGA

A fetus that is SGA exhibits biometric variables or a weight that deviates from the expected values whereas a growth restricted fetus is exposed to the pathological changes.

In investigations on infants born extremely preterm, the diagnosis of IUGR is seldom confirmed in a correct manner because of the young gestational age at birth. In our evaluation of survival and neonatal morbidity among infants born extremely preterm (Paper I), 16% of the 707 infants born alive were diagnosed as SGA. The proportion of SGA rose with increasing gestational age possibly because growth restriction had been present for a longer period of time. Since SGA infants run in their first month of life a higher risk for mortality than infants with normal weight, even after adjustment for GA, [121, 188] proper diagnosis of growth restriction in this cohort is crucial for optimal perinatal care.

As described above, in Sweden the diagnosis of SGA is based on a birth weight that is at least 2 SD less than expected for the gestational age. The Swedish birth weight standard is based on GA estimated by ultrasound measurement of BPD or CRL in 86 uncomplicated singleton pregnancies with a known date of LMP and a discrepancy between LMP and US less than 7 days. [151] Thus, this standard and thereby the diagnosis of SGA are based on the assumption that gestational age is estimated correctly by ultrasound and that there is no discrepancy in comparison to the gestational age estimates on the basis of LMP.

However, the discrepancy between GA based on LMP and mid-trimester ultrasound is associated with adverse neonatal outcome, e.g., low birth weight, and might indicate an early disturbance in fetal/placental development. [57, 188-190] In the EXPRESS registry, 16% of fetuses were older than 27 weeks when gestational age was estimated according to the LMP. (Paper II) The mortality rates were increased among these infants. The possible explanation of our findings in paper IV could be that the fetuses that were smaller than expected at the time of ultrasound scan, actually experienced IUGR and therefore had higher rates of neonatal death.

Since suspicion of IUGR is usually based on identification of an SGA fetus, which requires knowledge of its GA, calculation of this age can have a profound influence on making the correct diagnosis. Three compared ultrasonographic dating formulae had different rates of SGA and, as expected, the SGA rates were highest when gestational age was based on LMP (paper III). However, among pregnancies with discrepancy between GA according to the LMP and according to the Hadlock or Persson & Weldner formulae, both of formulae had elevated risk for SGA but there were no differences in OR between two of them. Thus, the prediction of SGA was not depending on the dating formula used for examination.

In conclusion, since a substantial proportion of the fetuses that appear to be SGA upon ultrasound examination are confounded by early fetal growth restriction, diagnosis of IUGR at the time of ultrasound examination would seem to be the more adequate than adjustment of the gestational age. In order to detect at time and confirm the diagnosis of IUGR, the fetuses that appeared smaller than expected at least 7 days at ultrasound examination in early pregnancy should be followed up by ultrasonographic fetometry and Doppler examination later in the pregnancy. Furthermore, an early dating of

pregnancy, e.g. at the time of Combined ultrasound and biochemical screening (CUB), may enhance the diagnosis of IUGR at the time of routine ultrasound scan at mid trimester.

6.3 CLINICAL IMPLICATION

Ethical decision making in the resuscitation of extremely preterm infants

The concept of viability is heavily emphasized in the neonatal and perinatal literature as a determinant in resuscitation decision making. [191, 192] Uncertainty about long-term prospects for infant´s health complicates the decision making.[193] To address the complexity of decision many professional societies and national commissions have developed policies or guidelines regarding treatment of preterm infants. [134, 194] The viability is important as it is used to define an acceptable age range in which resuscitation for infants at the extremes of prematurity is morally defensible. [195] However, when the likelihood of significant morbidity and poor quality of life for the infant is expected, then resuscitation needs to be questioned and reconsidered.[196, 197]

Probably one of the major reasons why neonatal policies might be different from the policies for older children or adults is the belief that gestational age is a more reliable predictor of prognosis than other clinical indicators. Numerous professional associations worldwide have very similar conclusions about resuscitation of extremely preterm born infants. At 22 weeks or less, a newborn is considered too preterm to survive and palliative care is recommended; at 23-24 completed weeks, prognosis is deemed so poor that life-saving interventions are considered optional and after 25 completed weeks life saving therapies are generally indicated. [59, 198-201] However, there are two reasons why gestational age does not yield reliable prognosis.

First, estimates of GA have rather wide margins of error. As described in this thesis, factors like maternal obesity, selection of dating formula as well as timing for the ultrasound examination can influence the gestational age estimates. These limitations on the accuracy of gestational age assessment have important implications if that assessment is being used to decide whether or not to withhold treatment. An assessed gestational age of 23 weeks and 5 days could in reality by anything from 22 to 25 weeks. The range of outcomes with such an interval of gestational ages is enormous.

Second, even if perfectly accurate, gestational age is only one component of reliable prognostication. Many other factors such as birth weight, sex, place of birth (tertiary vs. non-tertiary center) and prenatal treatment influence the prognosis. (Paper I) Adjusting for these factors fine-tunes the prognostic estimates made from gestational age alone. [91]

Given these many factors, guidelines that are based solely upon gestational age hopelessly oversimplify a complex situation. Resuscitation decisions for the extremely preterm infant should be approached in the same way as for other patients i.e. they should be individualized with objective and the most accurate individual prognostication, taking into account all of the relevant clinical characteristics. Families and health care providers asked to make critical decisions should be aware of uncertainty of gestational age estimation.

7 CONCLUSIONS

- Rates of survival to 1 year among infants born prior to 27 weeks of gestation in Sweden are comparatively higher than previously reported and rise with increasing gestational age at birth.
- Perinatal interventions such as tocolytic treatment, antenatal steroids, surfactant administration and birth at level III hospital reduce the risk of death up to age of one year among live-born extremely preterm born infants.
- Maternal obesity elevates the risk for fetuses to be smaller at ultrasound scan at mid-trimester than expected by calculation based on LMP.
- Available ultrasonographic dating formulae estimate gestational age differently but have similar potential in predicting small for gestational age fetuses
- Gestational age estimated on the LMP predicts in general longer pregnancies than ultrasound estimation but the survival and morbidity rates among extremely preterm born infants remain the same for the two methods.

8 POPULAR SCIENTIFIC SUMMARY IN SWEDISH POPULÄRVETENSKAPLIG SAMMANFATTNING

Handläggning av en graviditet och dess komplikationer baseras enligt dagens medicinska praxis oftast på beräknat förlossningsdatum, dvs. den aktuella graviditetslängden.

Omhändertagandet av hotande förtidsbörd och extremt för tidigt födda barn, dvs barn som fötts innan 27 graviditetsveckor, bygger på kunskap om riskerna med att födas för tidigt. Både vad gäller dödlighet och sjuklighet.

Sedan introduktionen på 1970-talet, har ultraljudsundersökning under graviditet blivit allt vanligare och idag genomgår drygt 95% av alla gravida kvinnor i Sverige en rutinundersökning med ultraljud kring 18 graviditetsveckor. Undersökningen syftar bl.a till att bestämma förlossningsdatum och därmed uppskatta den aktuella graviditetslängden. En korrekt bestämning av graviditetslängden har stor betydelse för extremt för tidigt födda barn, eftersom beslut avseende omhändertagande, liksom prognos och behandling grundas på graviditetslängden vid födelsen.

Avhandlingens syfte har varit att kartlägga överlevnad och sjuklighet hos extremt förtidigt födda barn, samt närmare undersöka faktorer som kan påverka ultraljudsbaserad datering av graviditeten och därmed utfallet under nyföddhets perioden.

Materialet för undersökningarna har hämtats från EXPRESS registret (Extremely preterm infant study in Sweden) som innehåller uppgifter om alla barn födda i Sverige före 27 fullbordade gravidtietsveckor under en treårsperiod, från 2004 till 2007. Totalt inkluderades 1011 barn, varav 707 var levandefödda och av dessa överlevde 70% till 1 års ålder. Överlevnaden bland intensivvårdade barn varierade från 26% vid 22 graviditetsveckor till 86% vid 26 graviditetsveckor. Bland de överlevande barnen uppvisade knappt hälften (45%) inga svåra komplikationer under nyföddhetsperioden, medan drygt hälften (55%) drabbades av en eller flera komplikationer såsom hjärnblödning, ögon- respektive tarmkomplikation, eller svår lungsjukdom. Åtgärder som var förknippade med högre 1-årsöverlevnad bland barnen var behandling av den gravida kvinnan med läkemedel som stillar värkarbetet och kortison som ges till modern för att påskynda fostrets lungmognad. Även snabbt insatta lungläkemedel (surfactant) till barnet efter födelsen hade en gynnsam effekt på 1-årsöverlevnaden. Den högre överlevnaden för dessa extremt för tidigt födda barn i Sverige jämfört med omvärlden, beror troligen på ett aktivt och gott omhändertagande av den gravida kvinnan, och en välfungerande intensivvård och god omvårdnad av barnen. Den starka korrelationen mellan överlevnad och graviditetslängd belyser vikten av korrekt beräkning av graviditetslängd för dessa barn eftersom handläggningen beror på graviditetslängden.

I EXPRESS registret var 95% av beräknade förlossningsdatum uppskattade med hjälp av ultraljudsmätningar av huvuddiameter och lårbenslängd, vilket följer gällande rekommendationer. För att omvandla fostrets mått vid ultraljudsundersökningen till en uppskattad graviditetslängd i dagar, används olika matematiska formler. I avhandlingen jämförs de tre mest använda formlerna, som har blivit namngivna efter deras upphovsmän: Persson, Hadlock och Mul. Vid en jämförelse av dessa tre beräkningssätt kunde vi konstatera att den beräknade graviditetslängden uppvisar signifikanta olikheter; vid en omräkning av graviditetslängderna enligt Hadlock och Mul visade det sig att gestationsåldern var högre än den ålder som registrerats i EXPRESS registret. Därmed kan graviditetslängd samt inklusionskriterier och resultat i vår studie vara beroende av valet av dateringsfomel som använts. Detta kan i sin tur försvåra jämförelser med internationella rapporter och studieresultat. Eftersom graviditetslängd i tidigare rapporter om extremt för tidigt födda barn, kan ha baserats på sista mensdata, undersöktes även hur denna metod kan ha påverkat resultaten i EXPRESS studien.
Vi kunde konstatera att graviditeterna i genomsnitt var längre när dateringen baserades på sista mens (SM) datum, men inga skillnader kunde påvisas i överlevnadsfrekvens eller sjuklighet i nyföddhetsperioden mellan dessa två metoder. Denna slutsats rättfärdigar jämförelse med andra studier.

Flera tidigare studier har visat att foster som är mindre vid ultraljudsundersökningen än förväntat enligt SM dateringen, löper högre risk att vara tillväxthämmade och att vara sjuka under nyföddhetsperioden.
Vi kunde i vår studie visa att överviktiga och obesa kvinnor löper högre risk för diskrepans mellan förväntat graviditetslängd enligt SM och enligt ultraljud. Studien baserades på 842 083 graviditeter med information om gravida kvinnor, deras vikt och längd samt information om ultraljudsmätningar och SM datum. Informationen inhämtades från Medicinska födelseregistret. Risken för att fostret skulle vara mindre och därmed att datumet för beräknad förlossning skulle senare läggas ökade med stigande maternell övervikt och fetma. Våra fynd kan ha biologiska förklarningar men troligtvis handlar det om tekniska begränsningar orsakade av moderns bukfetma.

Sammanfattningsvis har denna avhandling visat att dateringsformel och maternell fetma påverkar ultraljudsdateringen av en graviditet. Även val av metod för datering av graviditet påverkar beräkning av förlossningsdatum.Alla dessa faktorer bör beaktas vid omhändertagandet av extremt förtidigt födda barn. Vi kunde med hjälp av studierna i denna avhandling säkerställa att utfallet i den nationella kartläggning av extremt för tidigt födda barn som genomförts i Sverige inte påverkats av de faktorer vi studerat.

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