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Prenatal diagnosis of structural malformations and chromosome anomalies

Detection, influence of Body Mass Index and ways to improve screening

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LIST OF PAPERS

This thesis is based on the following original articles, which will be referred to in the text by their Roman numerals;

- I. Hildebrand E, Selbing A, Blomberg M. Comparison of first and second trimester ultrasound screening for fetal anomalies in the southeast region of Sweden. *Acta Obstet Gynecol Scand.* 2010 Nov;89(11):1412-9.
- II. Hildebrand E, Gottvall T, Blomberg M. Maternal obesity and detection rate of fetal structural anomalies. *Fetal Diagn Ther.* 2013;33(4):246-51.
- III. Hildebrand E, Abrandt Dahlgren M, Sved C, Gottvall T, Blomberg M, Janerot-Sjoberg B. Impact of a standardized training program on midwife's ability to assess fetal heart anatomy by ultrasound. Under review.
- IV. Hildebrand E, Källén B, Josefsson A, Gottvall T, Blomberg M. Maternal Obesity and risk of Down syndrome in the offspring. *Prenatal Diagnosis* 2013;33, 1-6.

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CONTENTS

List of papers.....	3
Contents.....	4
Abbreviations.....	6
Introduction	7
History of prenatal screening in Sweden	7
Prenatal diagnostics in Sweden today	8
Prenatal diagnostics in the southeast region of Sweden today.....	9
Screening for structural malformations.....	10
Congenital heart disease	11
Chromosomal anomalies.....	11
Factors affecting the performance of screening.....	14
Possible improvements in prenatal screening	14
Hypotheses	16
Aims of the research	17
Material and methods.....	19
Populations and participants.....	19
Registers and databases	19
Screening for structural malformations (Papers I and II).....	20
Examination of the fetal heart (paper III).....	23
Risk assessment for aneuploidy (paper IV)	29
Ascertainment of infants born with Down syndrome and risk associated with maternal obesity.....	29
Statistics.....	30
Ethics.....	30
Results	31
Detection of malformations and impact of obesity (papers I and II).....	31
Examination of the fetal heart (paper III).....	35
Detection of chromosomal anomalies and Impact of Obesity (paper IV)	37

Obesity as a putative risk factor for Down syndrome births	40
General Discussion	41
Discussion of the methodology	42
Discussion of the results	44
Conclusions	47
Future perspective of clinical implications	49
Summary in Swedish – Sammanfattning på svenska	50
Ultraljudsundersökning i Sydöstra sjukvårdsregionen.....	50
Screening för Missbildningar	50
Screening för Downs syndrom	50
Faktorer som påverkar screeningen.....	51
Syfte med studien.....	51
Metoder	51
Resultat.....	52
Acknowledgements.....	53
References	55
Errata	60

ABBREVIATIONS

AMC	Amniocentesis
β -hCG	Human chorionic gonadotropin beta
BMI	Body mass index
BPD	Biparietal Diameter
CHD	Congenital heart disease
CRL	Crown Rump Length
FL	Femur Length
FMF	Fetal Medicine Foundation, London
GA	Gestational age
IVC	Inferior Vena Cava
LA	Left Atrium
LPA	Left Pulmonary Artery
LV	Left Ventricle
MoM	Multiples of Medians
MRI	Magnetic Resonance Imaging
MV	Mitral Valve
NT	Nuchal translucency
NTD	Neural Tube Defect
PAPP-A	Pregnancy Associated Plasma Protein A
PV	Pulmonary Vein
RA	Right Atrium
RCOG	Royal College of Obstetricians and Gynaecologists
RPA	Right Pulmonary Artery
RV	Right Ventricle
SES	Socioeconomic status
SVC	Superior Vena Cava
TV	Tricuspid Valve
TOP	Termination of pregnancy
WHO	World Health Organization

INTRODUCTION

In Sweden nearly 2000 babies with major structural abnormalities or chromosomal anomalies are born every year. Finding these in early pregnancy is a challenge. The continuous evolution of the ultrasound equipment makes it possible to visualize the anatomical structures of the fetus in more and more detail. The ultrasound screening examination is most commonly performed in gestational week 18-20 to make it possible to identify structural malformations. At present, combined screening for aneuploidy is performed in gestational week 11-14 at many centers in Sweden. Fetal structural malformations could also be discovered at this earlier scan. However, the simultaneously increasing prevalence of obesity among the pregnant women is making ultrasound diagnostics more and more difficult. The competence of personnel and the need for training the personnel performing the scans are important factors determining the effectiveness of the screening.

HISTORY OF PRENATAL SCREENING IN SWEDEN

The first routine ultrasound was introduced in Malmö 1973, where all pregnant women were examined with ultrasound in gestational week 27 with the aim to detect twin pregnancies (Grennert 1978). About three years later the time of examination was adjusted and two examinations were offered. Gestational age was assessed at 19 gestational weeks and at 33 weeks intrauterine growth was evaluated by measuring the biparietal diameter (BPD)(Grennert 1978). The formulas for assessment of gestational age were developed in the mid-1980s, and many of these are still in use today (Hadlock 1984; Selbing 1985; Persson 1986).

Waldenström et al. confirmed that dating with ultrasound is more accurate than using the data on the last menstrual period if performed at 13-19 weeks of gestation (Waldenstrom 1988). The advantage of the assessment of gestational age in the first trimester, compared with the second trimester, was demonstrated by Anders Selbing in Linköping in 1983 (Selbing 1983). However at most centers the examination continued to be performed in gestational week 18-20, probably due to the low resolution of the ultrasound image.

It became evident that the possibility to detect fetal malformations gradually improved with the evolution of ultrasound systems with higher resolution.

Different biomarkers have been used to improve the prenatal diagnosis of some conditions. One example is the use of alpha-fetoprotein in the prenatal detection of

neural tube defects (NTD), already demonstrated in the early 1970s (Brock 1972). This method was effective but had a risk of false positive findings. Since the improved ultrasound image quality made it possible to reveal NTD the alpha-fetoprotein method is no longer in use in Sweden (Widlund 2007).

With the introduction of color-flow mapping in the 1980s the visualization of heart and vessels improved (DeVore 1987). Today, three-dimensional ultrasound (3D) and magnetic resonance imaging (MRI) are used as a supplement to 2D ultrasound in prenatal diagnostics (Yagel 2011; Paladini 2013). However, the two-dimensional gray scale ultrasound picture remains the basis of the routine screening situation.

In the 1980s soft-markers were introduced as a sign of an increased risk of a chromosomal anomaly, notably Down syndrome (Benacerraf 2010). Soft markers are sonographic features rather than malformations, indicating an elevated risk of a chromosomal anomaly. Since invasive testing for aneuploidy is associated with an increased risk of miscarriage, only women 35 years or older at the time of conception had the opportunity to have an amniocentesis made. With this method only 30% of the cases of Down syndrome could be detected, since the majority of infants with Down syndrome are being born by younger women. Another fact is that the proportion of pregnant women older than 35 is increasing, exposing more women to the risk of having a miscarriage as a consequence of the invasive test. The presence of soft-markers could be of some help, but the most important step towards the screening method used today was the association found between increased nuchal-translucency (NT) thickness and aneuploidy in pregnancy week 11-13+6 (Nicolaidis 1992). With the NT-test, a risk estimate could be calculated using maternal characteristics, measurement of NT-thickness and measurement of crown rump length (CRL) detecting about 70% of the fetuses with Down syndrome. Now it was time for a renaissance of important biomarkers in prenatal diagnostics. By adding two biomarkers from the placenta, Pregnancy Associated Plasma Protein-A (PAPP-A) and Human Chorionic Gonadotropin (β -HcG), and including these in the algorithm an approximately 90% detection rate of Down syndrome could be achieved (Kagan 2009).

PRENATAL DIAGNOSTICS IN SWEDEN TODAY

Examination of the fetus with ultrasound is now a well-established screening method offered to all pregnant women in Sweden with the aim to assess the gestational age, identify multiple pregnancies, identify the location of the placenta and detect fetal structural malformations. The screening is a part of the maternity health care system and >95% of the women used the opportunity to be examined (National Board 2004).

There are, however, no national guidelines for prenatal diagnosis in Sweden, and the ultrasound examinations offered vary in character. A screening ultrasound around gestational week 18-20 is the type most commonly offered to all pregnant women, whereas screening for chromosomal abnormalities with ultrasound is as yet offered in only 16 of the 21 counties in Sweden.

PRENATAL DIAGNOSTICS IN THE SOUTHEAST REGION OF SWEDEN TODAY

The Southeast region of Sweden consists of the counties of Östergötland, Jönköping and Kalmar (Figure 1). In the region approximately 11,000 babies are born every year. All pregnant women in the southeast region are offered screening with routine ultrasound examination as a part of the official maternity health care system.

A routine ultrasound examination in gestational week 11-14 was first introduced in Linköping and has been offered to all pregnant women since 1978. After the introduction of ultrasound in Linköping, screening ultrasound was gradually introduced for all women in the region. At all centers except Linköping and Eksjö the examination was performed in gestational week 18-20. The aim of the screening ultrasound was to assess gestational age, identify multiple pregnancies and assess the anatomy of the fetus.



Figure 1. The Southeast region of Sweden. The map is printed with permission from the Southeast region of Sweden.

Since 2009 the majority of centers in the region offer both first and second trimester examinations to all pregnant women and, from 2012 on, all centers have offered this combination. The Combined screening in week 11-14 is performed with both ultrasound and biochemistry in order to estimate the risk of carrying a fetus with a chromosomal anomaly. Assessment of gestational age (GA) and determination of chorionicity in case of a multiple pregnancy is also made. Screening for structural abnormalities is performed at 18-20 weeks of gestation including assessment of the site of the placenta. In general, the ultrasound examinations are performed by midwives specially trained in obstetric ultrasonography.

SCREENING FOR STRUCTURAL MALFORMATIONS

Structural malformations in the population occur in about 4%, of which 2% are regarded as major malformations (Saltvedt 2006). Frequencies of the most common fetal malformations are presented in table 1.

Table 1. Congenital malformations in children born and in fetuses in terminated pregnancies 1999-2011. Data from the Birth Defect Register in Sweden.

Diagnose	Number	Frequency/1000 children and fetuses
Anencephaly	422	3.26
Neural tube defects	531	4.10
Hydrocephalus	359	2.77
Orofacial clefts	1239	9.56
Cardiac defects	7801	60.18
Diaphragmatic hernia	315	2.43
Abdominal wall defects	451	3.48
Kidney ageneisi/dysgenesis	308	2.38
Cystic kidneys	406	3.13
Limb reduction defects	644	4.97
Pes equinovarus	1019	7.86

The reported overall sensitivity for the detection of structural malformations during routine screening ultrasound varies considerably between studies (Bricker 2000). The reported detection rate of fetal malformations in the second trimester screening varies between 19 and 80% (Boyd 1998; Eurenus 1999; Stefos 1999; Saltvedt 2006). The ability to detect structural malformations in the first and early second trimester

has improved so that detection rates (15-54%) are almost comparable to those from gestational week 18-20 (Carvalho 2002; Chen 2004; Taipale 2004; Cedergren 2006; Saltvedt 2006; Souka 2006). The ability to detect malformations also varies considerably between organ systems. Nakling et al reported a detection rate range from 8% to 74% depending on the organ system described (Nakling 2005). Anomalies of the urinary tract (74%) and the central nervous system (69%) were the easiest to detect whereas skeletal anomalies (8%) and cardiac anomalies (15%) are the most difficult to detect according to the Nakling study.

CONGENITAL HEART DISEASE

Cardiac defects, also defined as congenital heart disease (CHD), are the most common congenital defects and could lead to severe consequences for the child. CHD represents 25% of all major malformations in born infants (Allan 2000). Just below 1% of all fetuses are affected by CHD and usually there is no identifiable cause (Mitchell 1971; Cedergren 2003).

About half of the CHD cases are regarded as major, requiring surgery or intervention in the child's first year of life. Furthermore, about one third of the major CHD cases will have a duct-dependent anomaly, an anomaly that if not identified before birth or recognized shortly after birth will develop into a life threatening condition (Hoffman 2002; Tegnander 2006). At present, the most common way to assess the fetal heart in the screening procedure in Sweden is the visualization of the four-chamber view. It has been estimated that about 50% of the CHD could potentially be detected with this mode of view (Allan 2000).

The detection rate of CHD in different studies varies. In a large Swedish study (n=36,299) 15% of the major CHDs were detected (Westin 2006). A Norwegian study reported 46% detection rate at the time of the 18 week scan (Tegnander 2006). Del Bianco in Italy stated that almost 90% of the major CHDs were possible to detect. In this article the examinations were performed in week 20-24 (Del Bianco 2006). These data indicate that the screening performance in Sweden regarding CHD is in need of improvement.

CHROMOSOMAL ANOMALIES

With increasing maternal age fetal aneuploidy becomes more common (National Board 2004). The most common chromosomal anomaly is trisomy 21, also known as Down syndrome. Frequencies of the three most common chromosomal anomalies are presented in table 2. The average age of women giving birth in Sweden

has increased from 26.0 years in 1973 to 30.8 years in 2012. Despite the increasing average age of the women giving birth in Sweden, the rate of infants born with Down syndrome remains relatively constant (1/700-800 births). This is the result of the prenatal screening, where an increasing number of pregnancies are terminated due to aneuploidy.

Table 2. Chromosomal anomalies in children born and in fetuses in terminated pregnancies 1999-2011. Data from the Birth Defect Register in Sweden.

Diagnose	Number	Frequency/1000 children and fetuses
Trisomy 21/Down syndrome	3659	28.23
Trisomy 18	1006	7.76
Trisomy 13	384	2.96

In the combined screening, the risk of carrying a fetus with aneuploidy, notably Down syndrome, is estimated using the algorithm provided by the Fetal Medicine Foundation in London (Kagan 2009).



Figure 2. Measurement of the nuchal translucency (NT) thickness

With a combination of maternal age and characteristics, NT (Figure 2), β -hCG and PAPP-A a risk-estimate is calculated. Since the level of β -hCG and PAPP-A varies with gestational age, a specific multiple of the median (MoM) is calculated for every gestational age. The level of β -hCG and PAPP-A also varies with maternal weight. In heavier women the larger blood volume causes the level of the placental markers to be more diluted. Due to this the risk-estimate has to be adjusted for maternal weight (Spencer 2003). In the yearly audit of the prenatal screening in the Southeast region of Sweden, we have noticed that the β -hCG MoM and PAPP-A MoM tend to be less stable in the group of obese women compared to the MoM:s of the normal-weight women. This might have implications for the risk-assessment in the obese women (see figure 3 and 4).

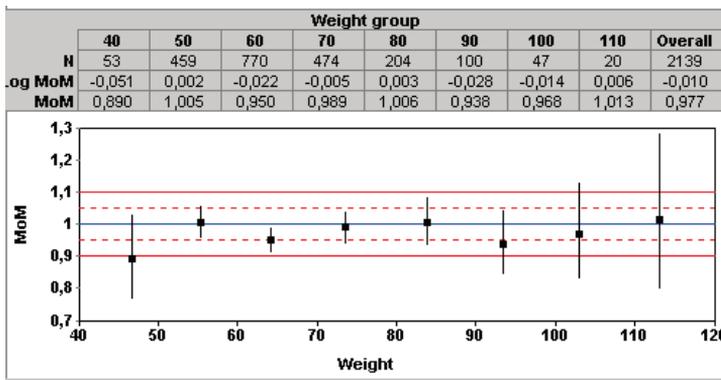


Figure 3: β -hCG MoM in different 10 kilo weight classes

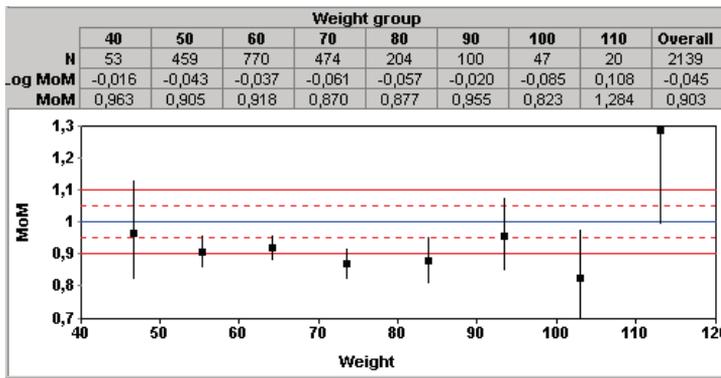


Figure 4: PAPP-A MoM in different 10 kilo weight classes

FACTORS AFFECTING THE PERFORMANCE OF SCREENING

Most of the scans in Sweden are performed by specially trained midwives in small ultrasound units at the local hospitals or maternity health care units. The need for continuous education is recognized as an important factor in ensuring effective screening that in principle is made possible by the improved resolution of the ultrasound image (Ogge 2006; McBrien 2010). The influence of obesity on diagnostic performance has not been studied extensively, but the rate of completed examinations was lower in the group with maternal obesity than in the other groups (Hendler 2004; Hendler 2004; Hendler 2005; Thornburg 2009; Maxwell 2010). The influence of maternal obesity on ultrasound detection of fetal anomalies has been studied by Dashe et al. (N=10,112) and Aagaard-Tillery et al (N=8,555) in two low-risk US populations (Dashe 2009; Aagaard-Tillery 2010). Both studies indicated a lower detection rate of anomalous fetuses among obese women compared to women with a normal BMI. The influence of a high body mass index (BMI) in detecting fetal structural anomalies by ultrasound in a low-risk population from a mainly rural setting in Sweden has not been evaluated.

It appears that maternal obesity also has implications in early pregnancy for the ultrasound measurement (Sahota 2009). NT was found to be thicker in the fetuses of obese women (Cowans 2011). Obesity also affects the level of the biomarkers used in the combined screening. In the algorithm described by the Fetal Medicine Foundation (FMF) in London adjustments in the measured maternal β -hCG and PAPP-A are made to adjust for maternal weight (Krantz 2005; Rode 2011).

POSSIBLE IMPROVEMENTS IN PRENATAL SCREENING

The actual overall detection rate of structural malformations and chromosomal anomalies when screening is performed in basic clinical care is unknown. Available studies describe a completely different context where screening is performed in high risk populations and performed by specialists in fetal medicine in tertiary centers. The actual detection rate at different gestational ages when screening is performed in basic clinical care is also unknown. If detection rates are lower in such settings, which is probable, one needs to think about what improvements could be developed and can realistically be implemented. Women should be aware of the effectiveness of the screening procedure at the specific clinic they will attend. Information could be published on the homepage of each maternal care unit for example.

Obesity is increasing dramatically worldwide and this emphasizes the need to evaluate the impact of obesity on the methods of prenatal diagnostics as it is not

satisfactory to offer a screening procedure that does not provide the same quality for both the thin and the obese. If the screening procedure results are affected by maternal obesity, each woman attending the screening examination must be informed properly concerning the effectiveness of her actual examination. Is it possible to perform an effective screening for fetal malformations in the presence of obesity? Is the risk assessment correct if performed in obese pregnant women? An evaluation of the present methods of prenatal diagnostics is important if we are to be able to inform obese women correctly and offer the best possible methods.

HYPOTHESES

- The detection rate of fetal malformation is higher if the ultrasound examination is performed in gestational week 18-20 compared to week 11-14.
- Maternal obesity is associated with a decreased possibility to detect fetal malformations.
- It is possible to improve the examination of the fetal heart with an intensive course and training of those doing the examination.
- Maternal obesity contributes to a less reliable risk-estimate in the combined screening for aneuploidy.

AIMS OF THE RESEARCH

- To assess the sensitivity of detecting structural fetal abnormalities and chromosomal anomalies by a routine ultrasound examination performed in the second trimester and to compare those results with results from the ultrasound examination at 11-14 weeks gestation in a low-risk population from a mainly rural setting in the southeast region of Sweden.
- To estimate the influence of high maternal BMI on the detection rate of fetal structural anomalies by a routine ultrasound examination either in the first or in the second trimester.
- To evaluate the possibility of introducing a more accurate fetal cardiac ultrasound screening based on five additional transverse views and color-Doppler by using a novel pedagogical approach to standardized cardiac imaging training.
- To determine if the risk estimate derived using an algorithm from the Fetal Medicine Foundation in London varies over BMI strata in the screening for Down syndrome and whether maternal obesity is associated with an increased risk of Down syndrome in the offspring.

MATERIAL AND METHODS

POPULATIONS AND PARTICIPANTS

In the Southeast region ultrasound examination during pregnancy is performed at the Departments of Obstetrics and Gynecology in eight different hospitals: Five hospitals participated: the University Hospital in Linköping (paper I-IV), Vrinnevi Hospital in Norrköping (paper I-II,IV), Ryhov County Hospital in Jönköping (paper I-II), Värnamo Hospital in Värnamo (paper I-III) and Motala Hospital in Motala (paper I-II,IV).

The majority of the examinations were performed by midwives specially trained in ultrasound examination. All midwives performing measurement of NT had attended the theoretical course supplied by FMF and submitted the images requested to receive the FMF Certificate of competence for the 11-13 weeks scan. Thirty minutes were allocated for each examination. The training necessary to perform the screening ultrasound in the second trimester comprised two compulsory courses, as well as practical training with an experienced midwife.

REGISTERS AND DATABASES

All ultrasound examinations performed are stored in the ultrasound application of the medical record database Obstetrix® (Siemens AB, Sweden) used for obstetrics in the region. In the beginning of the study period all hospitals had their own database, but during the last three years the counties have gradually created common databases for the hospitals in each county forming a total of three databases in the southeast region.

Another part of the Obstetrix® database contains the pediatric diagnoses set the first week after delivery, making it possible to identify children with structural malformations and chromosomal anomalies.

The database Obstetrix® contains no function for risk-assessment of chromosomal aneuploidy. For the risk-assessment a separate obstetrical database (Astraia®, Astraia software gmbh, Germany) is used where the algorithm supplied by FMF for risk-assessment of chromosomal aneuploidy is included. There are three Astraia®-databases in the region, one in each county.

The register on terminated pregnancies in Sweden does not include the personal identification numbers of the women whose pregnancies were terminated and could

therefore not be used for the purpose of these studies. In case of a malformation or a chromosomal anomaly leading to a terminated pregnancy the gynecological medical records have been manually scrutinized to confirm the suspected diagnosis.

The Medical Birth Register in Sweden contains data from the Maternity health care system, the delivery and the findings on the born child (National Board 2003). The external validity is high. Data from almost all (98-99%) children born in Sweden are reported continuously to the Medical Birth Register. The register is based on data from the standardized medical record forms completed at the prenatal health care centers at the start of the prenatal care usually in week 11 to 12, records from the delivery units and the pediatric examination of the newborn. From 1995 until June 2008 the register included stillbirths after 28 weeks gestation and from July 2008 until 2010 all stillbirths after 22 weeks. The internal validity varies depending on the variable studied. As an example, it was possible to calculate BMI for 86.7% of the pregnant women, for 13.3% of the women in paper IV data on height and/or weight was missing. A detailed description and validation of the register content is available (National Board 2003).

Two other registers were used as a complement to identify all cases of Down syndrome in paper IV. The Birth Defect Register, which includes reports from the cytogenic laboratories and the Hospital Discharge Register, a part of the Patient Register (National Board 2004; National Board 2012).

SCREENING FOR STRUCTURAL MALFORMATIONS (PAPERS I AND II)

The study population is described in figure 5. The second-trimester scans were performed between September 2001 to August 2004, and the first trimester scans were performed between September 2001 and April 2006. The contribution to the study population from each center is shown in figure 6. Measurement of biparietal diameter (BPD) and femur length (FL) at the second trimester scan and Crown Rump Length (CRL) at the first trimester scan were made to assess the gestational length. If any abnormality was suspected the woman was given a new appointment with a physician, and if needed referred to a specialist in fetal medicine at the University Hospital of Linköping. Only transabdominal scans were performed. If subsequent scans were performed during the pregnancy either due to poor visualization or other indications, the results of these additional examinations were not included in the study. For the purpose of this study, any structural anomaly detected at any other time during pregnancy was considered detected at birth. A local checklist for fetal

anatomy was used at some of the participating hospitals (table 3). No nation-wide checklist was available.

Table 3: The use of checklist to assess fetal anatomy at the five different units.

Linköping	Jönköping	Norrköping	Värnamo	Motala
No checklist	Head/CNS	Head/CNS	Head/CNS	No checklist
	Face	Heart (four chamber view)	Spine	
	Neck	Spine	Extremities	
	Spine	Diaphragm	Heart (four chamber view)	
	Extremities	Abdominal wall	Diaphragm	
	Thorax	Ventricle	Ventricle	
	Heart (four chamber view)	Kidneys	Kidneys	
	Diaphragm	Urinary bladder	Urinary bladder	
	Abdomen	Extremities	Abdominal wall	
	Ventricle/intestines	Feet		
	Kidneys	Hands		
	Urinary bladder			
	Abdominal wall			

The number of fetal structural anomalies diagnosed or suspected in the first week after birth was obtained from each unit. The unique maternal personal identification number was used to match the ultrasound examination with data on obstetric and neonatal outcome. Where no matches could be found (including all hospital in the region), the gynecological medical records were scrutinized to find information on miscarriage and/or termination of pregnancy. If available, results from the pathological anatomical examination or autopsy were extracted. All cases of miscarriage or termination of pregnancy due to a suspected anomaly were included in the study.

The diagnoses of structural malformations and chromosomal anomalies were grouped according to their likely clinical consequences as proposed by the Royal College of Obstetricians and Gynaecologists (RCOG) in 1997 (Whittle 1997). The sub-groups due to likely clinical consequences are: Major anomalies (lethal), anomalies associated with long-term handicap, anomalies potentially amenable to intrauterine treatment, and fetal conditions which require postnatal investigation and/or treatment. These sub-groups also include chromosomal anomalies, trisomy 21 as associated with long-term handicap and trisomy 13 and 18 as lethal.

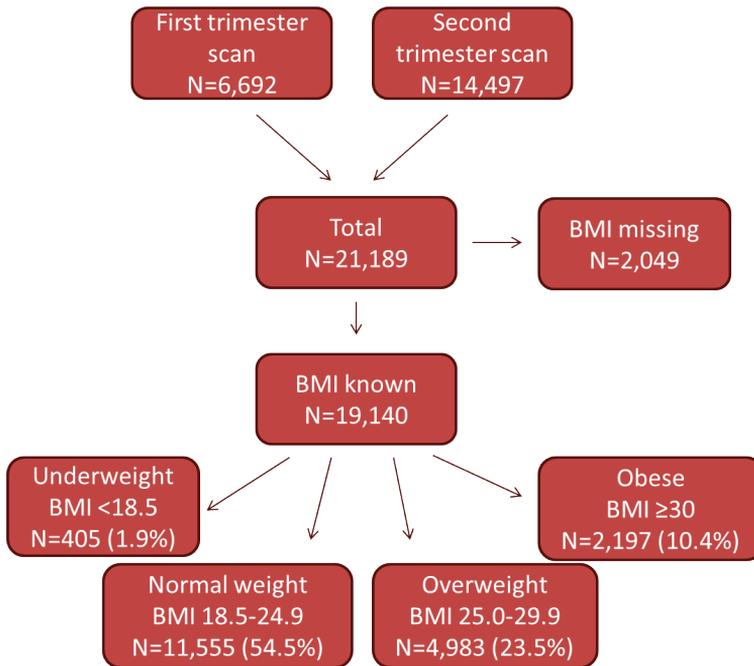


Figure 5. Flowchart of the study population (Paper I and II)

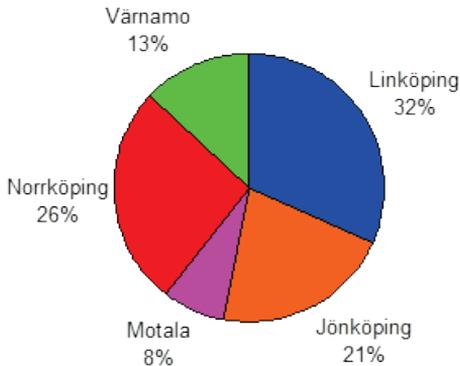


Figure 6. Contribution of each participating unit to the study population (Paper I and II)

The women were also divided into four BMI-groups according to the WHO guidelines; underweight ($<18.5 \text{ kg/m}^2$), normal weight (18.5 to 24.9 kg/m^2), over weight (25.0 to 29.9 kg/m^2), and obesity ($\geq 30.0 \text{ kg/m}^2$)(WHO 2000). For comparison a fifth group was constructed where data on the women’s BMI were missing. A power calculation was

done. We expected a detection rate of 20% in the group of obese women and 40% among normal-weight women, yielding a sample size of 20,250, which equals 80% power.

The detection rate of fetal structural malformations in obese women was compared with the detection rate in the group of women with normal pre-pregnancy BMI in the whole study population and in the second trimester scan group separately.

EXAMINATION OF THE FETAL HEART (PAPER III)

A course in standardized examination of the fetal heart and the use of color-Doppler was given at the University hospital in Linköping. The course was designed by a group of specialists in fetal medicine, pediatric cardiology and clinical physiology, a specialized sonographer, and a professor of education. Four midwives performing routine ultrasound screening in the southeast region attended the two-day course; two were from the university hospital in Linköping and two from the county hospital in Värnamo. Each hospital contributed one experienced midwife with more than 20 years of experience and one with maximum experience five years or less of ultrasound examinations.

At arrival, a written test was taken by the midwives. At this test, each midwife was individually presented a series of ultrasound recordings of the fetal heart as cine-loops in gray-scale with or without additional color Doppler. They were then equipped with dictaphones and instructed to “think aloud” by saying how they judged the scans and what they based their judgment on. They were also asked to comment on the quality of the scans and to note if the color Doppler added information. They were also asked to assess the scan as normal or pathological and how confident they felt about the judgment. The experiences from this test were then discussed (audio-recorded) by the midwives and the course leaders. Based on this discussion, a detailed course syllabus was created.

As a first step the midwives practiced color-Doppler examination of children and adolescents at the Department of Clinical Physiology. Each midwife had two hours of training on different patients. Informed consent was given by the patients and their parents.

On the second course-day, screening of the fetal heart was performed on pregnant women who had reached 20 weeks of gestation. Again, each Midwife had two hours of training on different fetuses after receiving informed consent from the women.

The midwives were taught how to record an ultrasound gray-scale-clip starting from the abdomen of the fetus and sliding in the cephalic direction. The different anatomical landmarks are shown in table 4. The examining technique was based on the five transverse views of the fetal heart as described by Yagel (Yagel 2001). The five different views are illustrated in figure 7-11 (Reprinted with permission from Tinytickers.org, UK).

The procedure with the ultrasound-clip was then repeated with color Doppler added. Color Doppler can be added in all views but is especially important in the four-chamber view and the three-vessel view, se figure 12-13.

Table 4. Protocol used for standardized examination of the fetal heart

1	Position of the fetus to determine the situs
2	Cross-section of the fetal abdomen, then slide in the cephalic direction
	S: Determine the position of the stomach, inferior vena cava and the aorta
	A: Four-chamber view
	B: Outflow-tract of aorta from left ventricle
	C: Outflow tract of pulmonary artery
	D: Three vessel view and arches
3	Repeat the slide with addition of color Doppler
	A: Four-chamber view
	B: Outflow-tract of aorta from left ventricle
	C: Outflow tract of pulmonary artery
	D: Three vessel view and arches

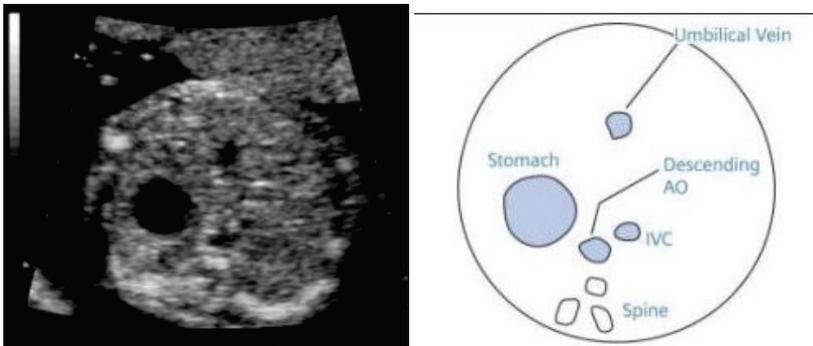


Figure 7. Abdominal situs. Shows the position of the descending aorta (AO), umbilical vein, inferior vena cava (IVC) and the spine.

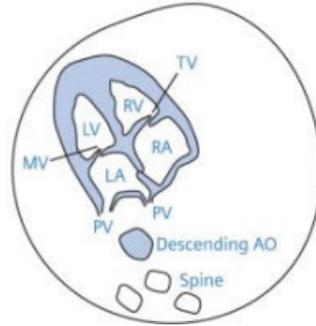


Figure 8. Four-chamber view. Showing the right and left ventricles (RV, LV), the atria (LA, RA), the descending aorta, the mitral valve (MV), the tricuspid valve (TV) and the pulmonary veins (PV) entering the left atrium.

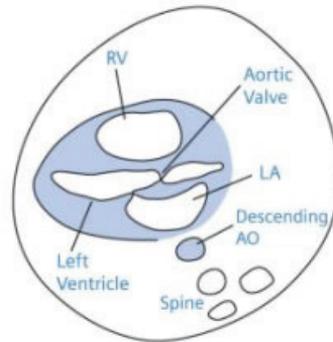


Figure 9. Left outflow tract of the heart.

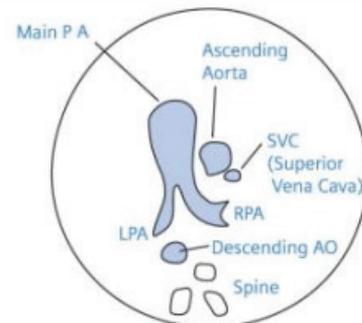


Figure 10. Right outflow tract from the heart. Pulmonary artery with main branching pulmonary arteries, the descending and ascending Aorta.

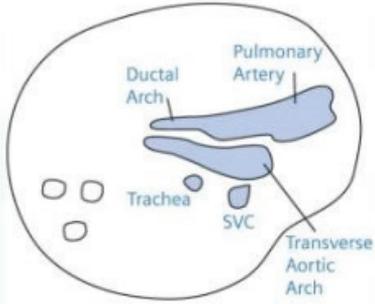


Figure 11. The three vessel-view and arches.

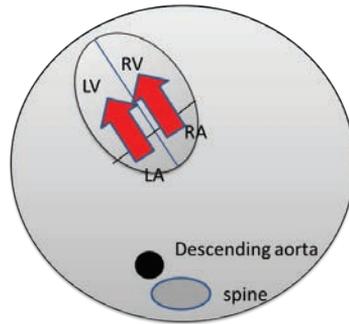
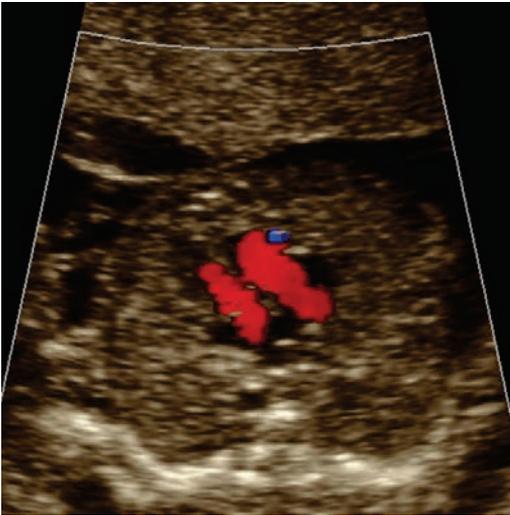


Figure 12. Color-Doppler of the blood flow filling the ventricles of the fetal heart. The red color indicates the direction of the flow towards the ultrasound probe, and in this case the apex of the heart.

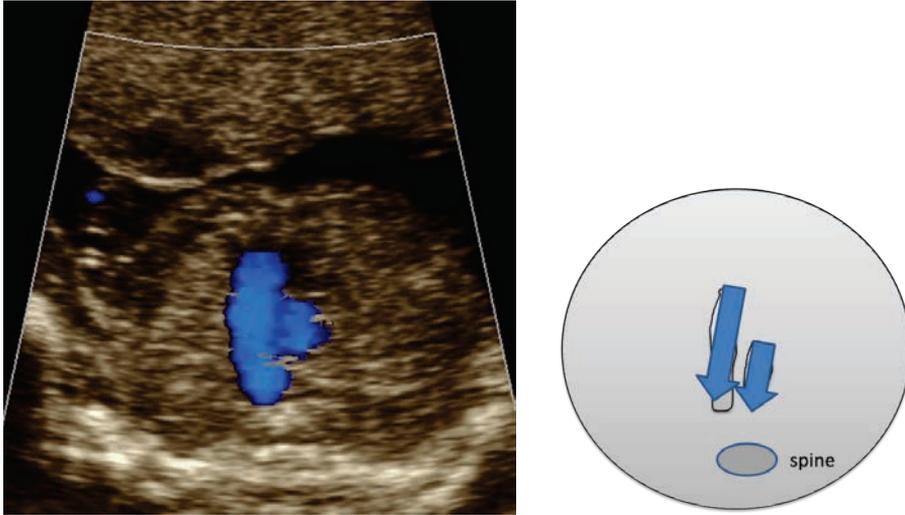


Figure 13. Color-Doppler of the blood flow of the pulmonary artery and the aorta. The blue color indicates the flow-direction away from the ultrasound probe, and in this case the correct flow-direction out of the heart.

With these experiences a second group discussion session was held (audio-recorded) for later analysis. The courses ended by having the midwives take the same test that they took before starting the course.

Before leaving, the midwives were given a protocol to be used in their clinical practice until the first follow-up. This protocol included documentation of their self-assessed ability to perform the different steps in assessing the fetal heart (table 5). The self-assessed significance of the use of color Doppler was also documented (table 6). Recordings of all these examinations were stored in the local databases, and thereby available for a second review. The time used to achieve the proper projections of the fetal heart was noted in the protocol.

Table 5. Self- assessed confidence to perform the method in clinical praxis

1	Uncertain
2	More uncertain than certain
3	More certain than uncertain
4	Certain

Table 6. The Significance of color Doppler for assessment of the heart anatomy

1	No significance
2	Little significance
3	Great significance
4	Crucial

The recordings from the group interviews were transcribed and then analyzed qualitatively, using a simple content analysis to describe the midwives’ reflections on the use of this new technique. The method of content-analysis was inspired by the methodology described by Graneheim et al. (Graneheim 2004).

The ultrasound recordings from the first and last 10 exams during the three month period in clinical use after the course were reviewed and assessed by a specialist in fetal medicine and by a cardiac sonographer specialist together. The ability of the midwife to perform the different steps in the examination was noted in a protocol.

Two years after the primary course, a second follow up was performed. The same four midwives documented another 10 ultrasound examinations. The follow up included the self-assessment concerning confidence in using the method (Table 5-6) and a review of the ultrasound recordings by the same specialists who did the primary evaluation. Additionally, the midwives took the test identical to the pre-course test described above, where a series of ultrasound recordings were assessed, this time without dictaphones. The flow of the training program is presented in figure 14.

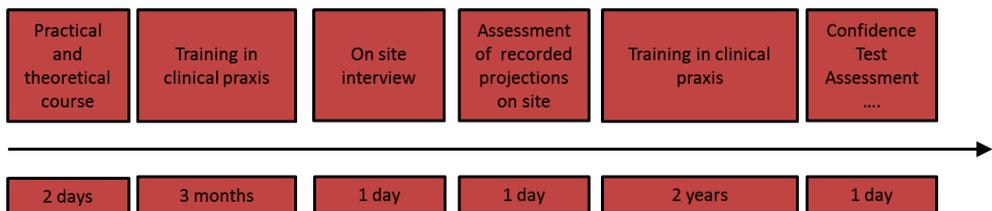


Figure 14. Flow chart of the training program

RISK ASSESSMENT FOR ANEUPLOIDY (PAPER IV)

The Astraia[®] database in Linköping was used to extract data from the risk-assessment of fetal aneuploidy from April 1 2009 until Dec 31 2011. The population consisted of 10,224 women who had undergone prenatal screening for aneuploidy, using an algorithm employing a combination of maternal age, NT, β -hCG, PAPP-A based on the formulas derived by FMF (Spencer 1999; Spencer 2003; Avgidou 2005; Nicolaides 2005; Kagan 2008; Kagan 2009). In the algorithm the estimates of PAPP-A and β -hCG were adjusted for maternal weight (but not BMI). The variables extracted were: maternal weight, calculated risk for trisomy 21, gestational day of the ultrasound examination, NT thickness, maternal age at the ultrasound examination, PAPP-A MoM, and free β -hCG MoM. During the study period women with a risk for trisomy 21 greater than 1/300 were offered invasive testing.

Data on maternal height for the calculation of maternal BMI were not available in the Astraia database and were therefore retrieved from Obstetrix[®] database and linked to the Astraia data set using the maternal personal identification. To investigate the effect of BMI on the risk-assessment the population from the Astraia[®] database was used divided into the six BMI-classes according to the WHO guidelines; underweight (<18.5 kg/m²), normal weight (18.5 to 24.9 kg/m²), over weight (25.0 to 29.9 kg/m²), obesity class I (30.0 to 34.9 kg/m²), obesity class II (35.0 to 39.9 kg/m²) and obesity class III (\geq 40 kg/m²) (WHO 2000) .

Outcomes estimated were risk assessments for Down syndrome, NT thickness, PAPP-A MoM and free β -hCG MoM. The cases with a risk-estimate $>1/300$ where most of the cases of aneuploidy could be expected were excluded to avoid bias of a potential association between maternal BMI and Down syndrome.

ASCERTAINMENT OF INFANTS BORN WITH DOWN SYNDROME AND RISK ASSOCIATED WITH MATERNAL OBESITY

To analyze the possible association between maternal obesity and Down syndrome a population from the Swedish Medical Registers was used. To find the infants with Down syndrome three sources were used: the Medical Birth Register, the Birth Defect, and the Hospital Discharge Register. The study population was divided in the six BMI-classes suggested by WHO (details above). Normal weight women were set as reference and all other BMI groups were compared to them in order to evaluate if there was an association between maternal overweight, maternal obesity and Down syndrome births.

Data on 1,568,604 women from the register who had given birth in Sweden from January 1, 1995 through December 31, 2010 were included.

STATISTICS

Statistical analyses for paper I-III were performed using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA; 2003, version 16.0). The statistical significance of differences in proportions was determined using the chi-square test. Logistic regression was used in order to detect differences between the different BMI categories. A two-sided P value of <0.05 was considered statistically significant. For paper IV non-parametric Mann-Whitney is used for univariate analysis of the time used to achieve the right views of the heart in praxis.

In paper IV medians with 95% CI were estimated using Analyze-it software (Analyze-it Software, Ltd, Leeds, UK). In order to compare risk estimates from the combined screening in different BMI classes, the Wilcoxon non- parametric method was used because of the extreme skewness of data. For each maternal age class (one year) the rank sums of cases in a specific BMI class were compared with the rank sum of the reference BMI class (BMI 18.5-24.9) and the rank sum T of the former was determined with its error. The mean for these T values with its error was compared with zero. Zero would indicate no difference.

Maternal age (1-year classes) is an important confounding factor for the data from the Swedish Medical Birth register, and was included in the adjusted analyses. Adjusted odds ratios (OR) were determined using the Mantel-Haenszel technique (Mantel N 1959). Estimates of 95% confidence intervals (95%CI) were made with a test-based method, based on the Mantel-Haenszel chi-square (Mantel N 1959)

ETHICS

Papers I and III were not considered by the ethical review board. Due to the design they did not meet the criteria of the review board at the time of the implementation of the studies. For papers II and IV, the Regional Ethical Review Board in Linköping has approved the study (2005/M44-09 and 2013/4-31).

RESULTS

DETECTION OF MALFORMATIONS AND IMPACT OF OBESITY (PAPERS I AND II)

The number of fetuses and babies with structural abnormalities in the study population was 858 out of 21,189 (4%) and 421 out of 21,189 (2%) was regarded as at least moderate. Mean maternal age at time at conception was 29.3 (range 15 – 48) years (figure 15). The spectrum of maternal age was similar among the participating units. Maternal age in Linköping (11-14 week scan), was slightly higher, mean 29.7 (range 15-46) years, compared with the mean at other units of 29.1 (range 15-48) years ($P < 0.05$).

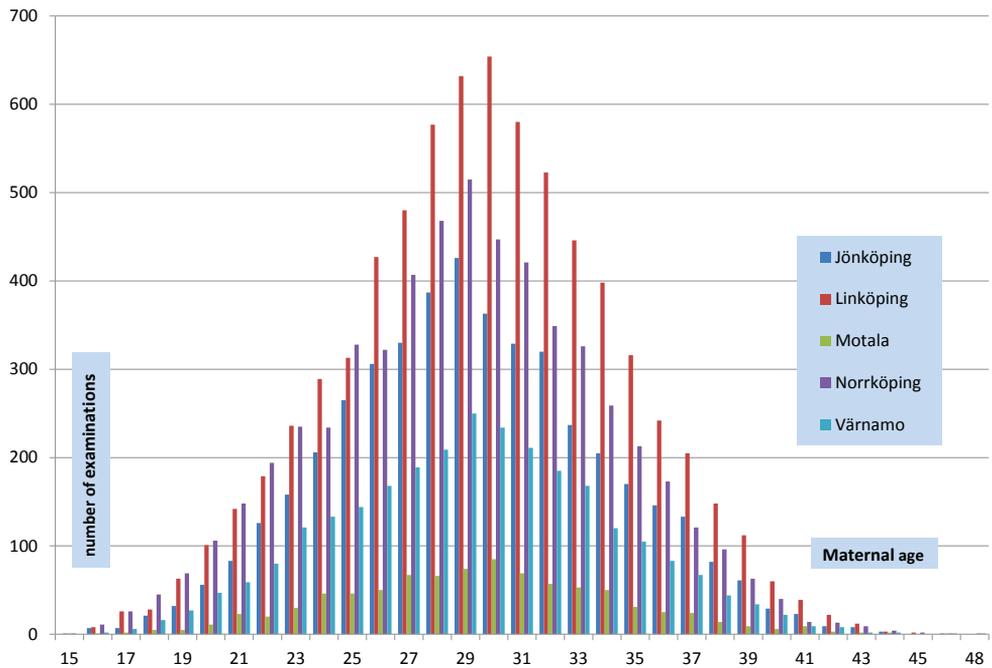


Figure 15. Maternal age at conception.

Gestational age (GA) at screening is presented in Figure 16. The scans were performed slightly later in Jönköping in comparison with Motala and Norrköping ($P < 0.05$). Data on GA at screening were not available from Värnamo. At one of the units (Norrköping) the majority of GA was reported every other day. This could be due to measuring BPD in whole millimeters instead of tenths of millimeters.

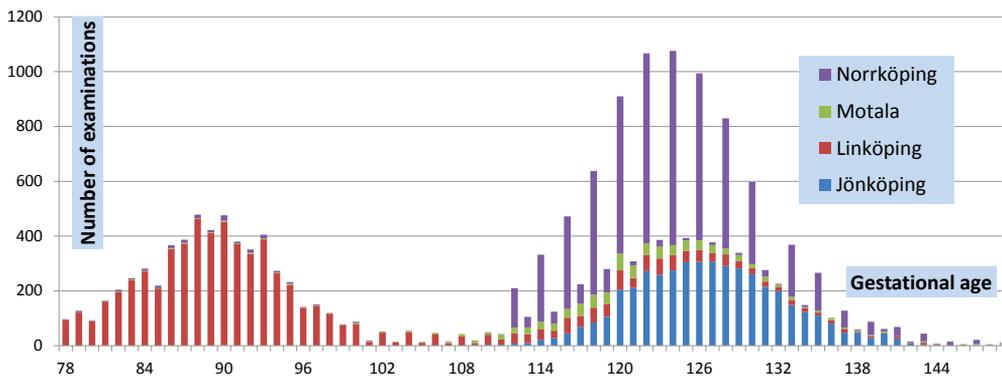


Figure 16. Gestational age at ultrasound examination.

In the 11-14 week scan group, 15 of the 120 structural abnormalities were detected in the routine scan situation (13 %) compared to 75 out of 261 (29%) in the second-trimester. Anomalies associated with long term handicap showed a detection rate of 35% (12 out of 34) in the early-scan group compared to 44% (35 out of 79) in the second-trimester. The detection rate of lethal anomalies was 88% (seven out of eight) and 92% (12 out of 13) in the two groups respectively. Chromosomal anomalies were discovered at 71% in the early scan, and 42% in the second trimester. Table 7 shows the antenatal detection rates of congenital anomalies according to organ system.

Table 7. Detection rates of congenital anomalies according to organ system (TOP Termination of pregnancy)

	First trimester				Second trimester				TOP
	Discovered at USG	% at USG	Discovered at birth	% at birth	Discovered at USG	% at USG	Discovered at birth	% at birth	
CNS	9	52.9	8	47.1	16	84.2	3	15.8	25
PULM	1	100.0	0	.0	2	40.0	3	60.0	3
CARDIAC	0	0	62	100.0	5	5.4	88	94.6	1
GI	3	27.3	8	72.7	7	36.8	12	63.2	7
URINARY TRACT	0	.0	5	100.0	28	52.8	25	47.2	10
SKELETAL	0	.0	13	100.0	8	18.6	35	81.4	2
OTHER	2	18.2	9	81.8	9	31.0	20	69.0	10
Subtotal	15	12.5	105	87.5	75	28.7	186	71.3	58
Trisomy 13					2	66.7	1	33.3	1
Trisomy 18	1	50.0	1	50.0	4	80.0	1	20.0	5
Trisomy 21	4	66.7	2	33.3	4	25.0	12	75.0	8
Other chromosomal	5	83.3	1	16.7	1	50.0	1	50.0	5
Subtotal	10	71.4	4	28.6	11	42.3	15	57.7	19
TOTAL	25	18.7	109	81.3	86	30.0	201	70.0	77

The most common false positive finding was hydronephrosis >5 mm at screening in the second trimester. Of the 68 cases discovered, 49 normalized during pregnancy or after delivery. Seven cases of amniotic band were also noted at the second trimester scan, but these did not affect the fetus or born child. The most common finding in the first trimester was increased NT, resulting in a number of amniocenteses. The false positive findings are presented in table 8.

Table 8. False positive findings at the second trimester screening.

Diagnosis	Trimester	Number of false positive findings	Intervention
Hydronephrosis	II	49	1 AMC
Amniotic band	II	7	-
Small BPD	II	1	-
Echogenic bowel	II	1	1 AMC
Choroid plexus cyst	II	7	6 AMC
Unilateral hydrocephalus	II	1	-
NT > 3 mm	I	15	14 AMC

The percentage of obese women was 10.4% (n=2,197), over-weight 23.5% (n=4,983), normal weight 54.5% (n=11,555) and underweight 1.9% (n=405). Among the obese the BMI-range was 30.0 – 54.3. When subdividing the obese group 7.3% (n=1,554) were in obesity class I, 2.2% (n=475) in obesity class II and 0.8% (n=168) in obesity class III.

The detection of fetal malformations for underweight women was 20% (2/8), normal weight women 26% (57/161), overweight women 29% (29/71) and obese women 19% (8/34). Anomalies associated with long term handicap were detected in 46% of normal weight women, 51% of overweight women and in 27% in the obese women. Corresponding data for the examinations performed in the second trimester were 30% for normal weight, 36% for overweight, and 21% for obese women, and for anomalies associated with long term handicap 51%, 57% and 29%. The odds ratios with 95% confidence intervals comparing detection rates between underweight, overweight and obese women to normal weight women for the second trimester are shown in table 9.

Table 9. Structural anomalies identified at the first screening scan in the first or second trimester and further classified according to severity presented in different maternal BMI groups. Odds ratios with 95% confidence intervals were estimated with normal weight women as reference.

Anomalies	N	<18.5	18.5-24.9	25.0-29.9	≥30	Data missing
Lethal	28	-	1.0	-	-	-
Long term handicap	137	-	1.0	1.23 (0.55 - 2.74)	0.44 (0.11 - 1.79)	0.50 (0.17 - 1.45)
Potentially amenable to intrauterine therapy	3	-	1.0	-	-	-
Requiring postnatal investigation.	278	2.93 (0.54 - 15.85)	1.0	1.47 (0.61 - 3.55)	1.30 (0.40 - 4.24)	2.30 (0.81 - 6.53)
Total	421	0.71 (0.15 - 3.42)	1.0	1.15 (0.68 - 1.95)	0.67 (0.29 - 1.52)	1.18 (0.60 - 2.31)

EXAMINATION OF THE FETAL HEART (PAPER III)

The two day course in examination technique of the fetal heart was evaluated with both quantitative and qualitative methods.

Quantitative results from the 2-day course: The results of the written tests performed at the beginning and at the end of the two-day course in examination technique of the fetal heart were similar, 23/132 vs 21/132 incorrect answers (ns). The self-assessed uncertainty was lower at the post-course test compared to at the beginning; 30/132 vs 11/132. ($p=0,006$).

Qualitative evaluation of the interviews: The recorded group discussions prior to the training showed that the use of color Doppler produced unfamiliar images that some of the midwives found difficult to interpret. This was also commented on in the think-aloud protocols of the pre-course test. The training seems to have enhanced the familiarity with the new technique. The importance of the hands-on training was emphasized as being critical for learning. The judgment of the scans only, as performed in the pre-course test, seemed to deprive the midwives of some of the manual skills in identifying anatomical landmarks and orientation needed to perform the ultrasound. Table 10 illustrates the four midwives' judgments of 33 scans at the pre- and after-course test. Each scan consisted of several views, making several judgments possible. During three months, 80 examinations were performed with the new method, each midwife contributing with 20 examinations. The results are presented in table 11. A correct scan had to include all the steps described in the method.

Table 10. Midwives' judgments of 33 scans before and after the course. Several judgments possible per scan.

Judgments	Before training	After training
No judgment made	28	29
Judgment without motivation	17	13
Judgment with motivation based on >1 view	33	36

Quantitative results from the 3 months follow up: There were large inter-individual differences in success-rate of correctly performed recordings ranging from 20/20 to 9/20 and the differences were associated with the experience level of the midwife.

Quantitative results from the 2-year follow up: Two years after the course the same midwives recorded 40 new ultrasound examinations of the fetal heart, 10 by each midwife. The results are presented in table 11. Results from the written test were in accordance with the results from the pre- and after-course tests (ns), 19/132 incorrect answers and the self-assessed uncertainty was 17/132.

Table 11. Self-assessment of the examinations in clinical praxis, number of correct performed examinations when assessed by specialist in fetal medicine and specialized sonographer and time spent to achieve the proper projections.

	At three months	At two years	p-value
Self-assessed confidence to perform the method, median/range	3/(1-4)	4/(2-4)	-
Self-assessed significance of color-Doppler, median/range	2.5/(2-4)	3/1-4)	-
Self-assessed correctly performed examinations (grey scale)	56/77 (73%)	33/40 (83%)	0.240
Self-assessed correctly performed examinations (color Doppler)	53/77 (69%)	32/40 (80%)	0.199
Specialist review: Correctly performed gray-scale examinations	67/80 (84%)	30/40 (75%)	0.251
Specialist review: Correctly performed color-Doppler examinations	52/80 (65%)	33/40 (83%)	0.047
Time spent to achieve the proper projections median/range (min)	4 (3-10)	3 (2-5)	<0.01

DETECTION OF CHROMOSOMAL ANOMALIES AND IMPACT OF OBESITY (PAPER IV)

In the group of women accepting screening for fetal aneuploidy from April 1 2009 until Dec 31 2011 (n=10,224) the prevalence of obesity was 12.9%. The corresponding rates for obesity class I-III were; class I 9.0%, class II 3.0% and class III 1.0%. The risk estimates for Down syndrome show an extremely skewed distribution. Women with a risk estimate for trisomy 21 of $>1/300$ were excluded. With increasing BMI there is a trend of increasing risk-estimate. Underweight women had the lowest risk for Down syndrome and morbidly obese women the highest (figure 17).

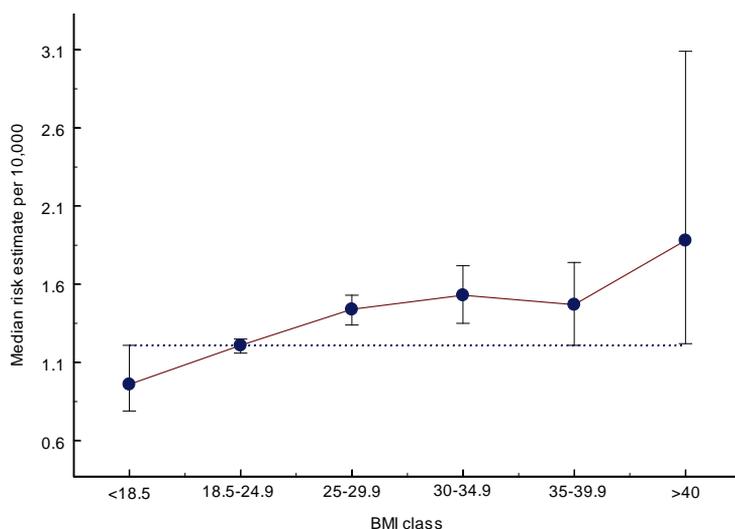


Figure 17: The risk for trisomy 21 Median risk for trisomy 21 with 95% confidence intervals according to woman's BMI. Women with a calculated risk $>1/300$ were excluded. Horizontal line shows mean for the normal BMI group (18.5-24.9).

The mean measured NT thickness increased with increasing maternal BMI (Figure 18), without adjustment for maternal age and with exclusion of women with a calculated risk of $>1/300$. There was no significant difference in gestational age at examination between the different BMI-groups.

Weight-adjusted levels of PAPP-A MoM and free β -hCG MoM are used in the formula for calculating the risk of Down syndrome. When comparing the levels between the maternal BMI strata (Figure 19) no certain effect was seen for PAPP-A but a tendency to decreasing β -hCG with increasing BMI was observed. Again, women with a calculated risk of $>1/300$ were excluded.

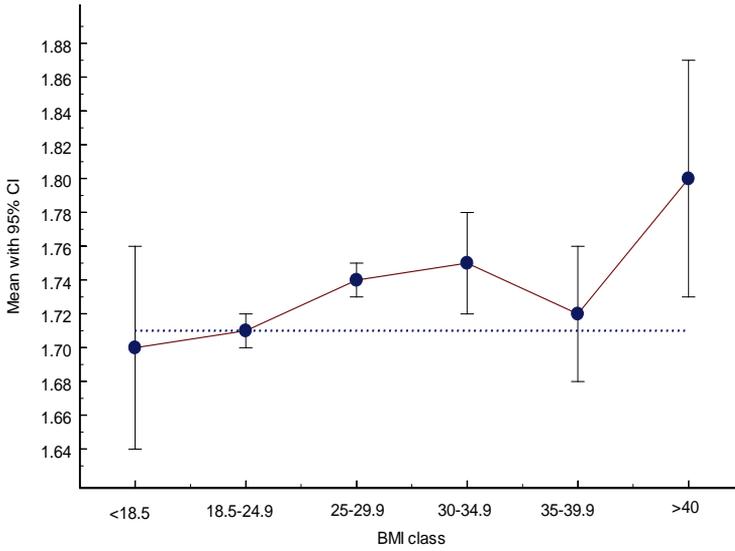


Figure 18: Means of nuchal translucency thickness (mm) according to BMI class. Cases with a trisomy 21 risk >1/300 were excluded. Horizontal line shows mean for the normal BMI group (18.5-24.9).

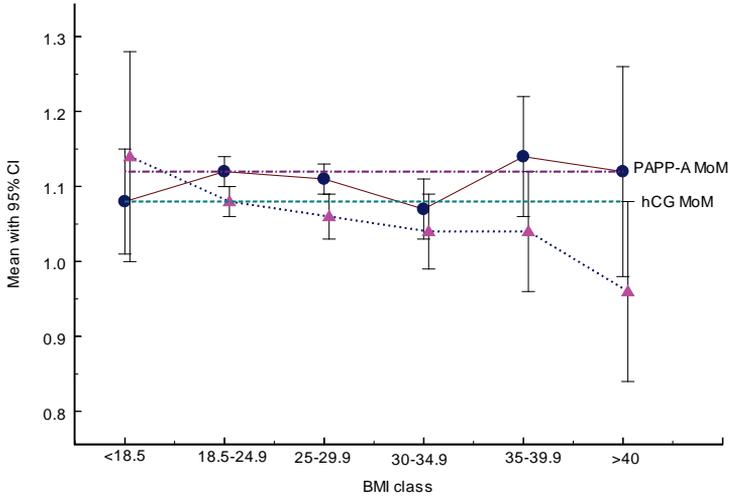


Figure 19: Means with 95% CI of PAPP-A MoM and hCG MoM for different BMI classes. Horizontal lines show values from normal BMI (18.5-24.9).

Since the risk for trisomy 21 increases with woman's age and the BMI distribution also varies with age, adjustment for maternal age was made. Figure 20 shows the mean risk estimate for each maternal age (1-year) for the normal BMI class (18.5-24.9) and for overweight and obesity class I and II+III. A clear-cut difference is seen for obese women at ages greater than 35 years.

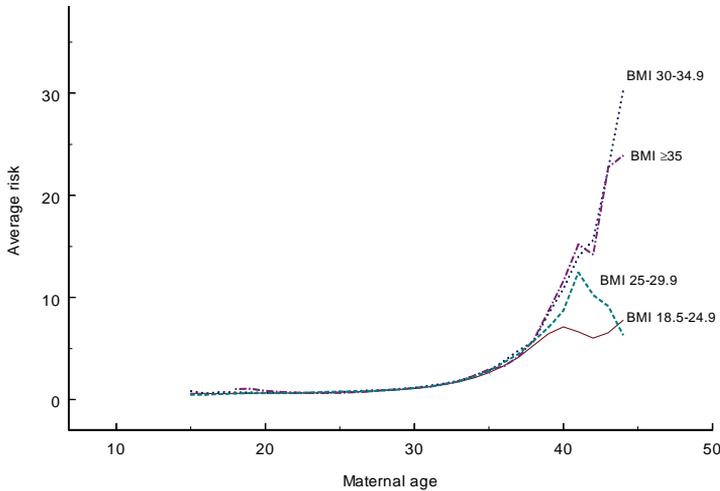


Figure 20: Mean trisomy 21 risk in the first trimester per 10,000 according to maternal age (1 year class) for different BMI classes (moving averages)

In order to find out if these maternal BMI effects were statistically significant, we used the Wilcoxon non-parametric test and compared the risk estimate for obese women and normal-BMI women with a T-test (rank order test) within each maternal age. We calculated the mean T value for all ages and compared with 0 which was the expected value if no difference existed. For BMI group 30-34.9, mean $T = 0.81 \pm 0.28$, $z = 2.89$, $P = 0.006$) and for BMI group ≥ 35 mean $T = 0.40 \pm 0.27$, $z = 1.50$, $p = 0.13$, thus not statistically significant, but for both BMI classes the mean T was positive, that is, there was a higher risk for obese women than for women with normal BMI.

The limit above which amniocentesis was offered during the study period was a risk for trisomy 21 higher than $1/300$. The percentages of women in each BMI class with a risk estimate $>1/300$ differed according to BMI class (table 12). Since the age distribution differed between the BMI classes, we calculated the expected number of cases in each BMI class, adjusting for maternal age (one year class). The expected number of cases with a risk of $>1/300$ within each BMI class was thus estimated from the age-specific risk for Down syndrome multiplied with the number of individuals in that BMI and age class.

Table 12. Number and percentage of women with a risk of Down syndrome >1/300 for each BMI class after adjustment for maternal age. Calculated expected number of cases due to maternal age is shown as a comparison.

BMI class	Number >1/300	Total number	Per cent >1/300	Expected Number >1/300
<18.4	8	206	3.9	7.4
18.5-24.9	286	5899	4.1	285.0
25-29.9	154	2799	5.5	156.5
30-34.9	53	918	5.7	52.8
35-39.9	18	304	5.9	13.9
≥40	8	98	8.2	7.6

OBESITY AS A PUTATIVE RISK FACTOR FOR DOWN SYNDROME BIRTHS

The overall crude risk of having an infant with Down syndrome among women giving birth in Sweden from January 1, 1995 through December 31, 2010 was 1.31 per 1000 births.

The prevalence of obesity (BMI ≥ 30) was 10.6% distributed in obesity class I-III as follows: class I 7.6%, class II 2.2% and class III 0.8%. Obese women (BMI ≥30) had a greater risk for giving birth to an infant with Down syndrome than did normal-weight women, OR 1.28 (95%CI 1.12-1.48) after adjustment for maternal age (one year classes) (Figure 21).

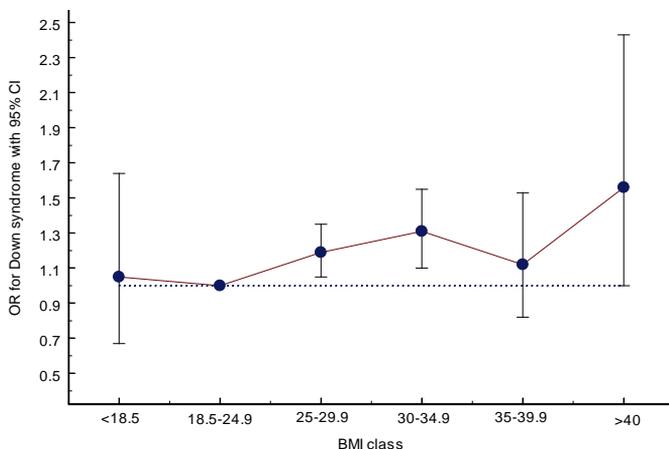


Figure 21: The risk of Down syndrome births. Odds ratio (OR) with 95% confidence interval (95% CI) for Down syndrome at different BMI classes, adjusted for maternal age (one-year). Horizontal line shows reference, normal BMI.

GENERAL DISCUSSION

Detection of structural fetal abnormalities and chromosomal anomalies

The sensitivity of detecting structural fetal abnormalities was significantly higher in the second trimester (29%) compared to the first trimester (13%). Of the lethal abnormalities, 92% were found in the second trimester and 88% in the first trimester (n.s.), and abnormalities that may cause future handicap 44% vs 35% (n.s.). There were advantages with regard to aneuploidy at the first trimester scan (71% vs 42%) despite no formal measurement of NT.

Influence of a high maternal BMI on detection rate of fetal structural anomalies

Detection rate of fetal structural anomalies for underweight women was 20%, normal weight women 26%, overweight 29% and obese 19% (I and II trimester).

Corresponding figures for the second trimester showed the same pattern with 29%, 30%, 36% and 21% respectively. In absolute numbers it seems as if maternal obesity decreases the possibility of detecting anomalies with long term handicap although the small number requires that care must be taken when interpreting data.

Evaluation of the possibility of introducing a more accurate fetal cardiac screening by using a novel pedagogical approach to imaging training

It seems that by participating in a continuous well-structured training program the competence of the midwives could become able to detect fetal cardiac defects in a more effective way and ensure the optimal care for the affected children after birth but these encouraging results must be further evaluated by including a larger number of midwives and long-term follow up of CHD detection rates.

Association between maternal obesity and Down syndrome in the offspring and the variation of the risk estimate over BMI-strata from the combined screening

The risk of giving birth to an infant with Down syndrome was 28% higher among obese women compared to normal weight women after adjustment for one-year maternal age. The prenatal screening procedure for Down syndrome with combined ultrasound and biochemistry offered to women in the first trimester seemed equally effective irrespective of maternal BMI.

DISCUSSION OF THE METHODOLOGY

We used the classification by the RCOG for the fetal anomalies. The classification reflects the likely clinical consequences. However, other definitions of anomalies exists which sometimes make it difficult to compare the results between studies (Boyd 1998).

To build the database including both the findings at the ultrasound examination and the outcomes of the pregnancies, several sources of information had to be used. The results of the ultrasound examinations had to be manually assessed and stored in a dataset. The unique personal identification number was then used to match the examination with the neonatal outcome. If there was no match, the gynecological medical records were scrutinized to find information on miscarriage or termination of pregnancy. In a following step, results from pathological anatomical examination or autopsy were extracted. This process was time consuming. Better systems for documentation would facilitate continuous quality control and we therefore started the work on making improvements directly after completing the study.

In study I, the size of the study-population and the follow-up of 89% of the consecutive ultrasounds are acceptable. However, there are several shortcomings. The ultrasound examinations in gestational week 11-14 were all performed at one ultrasound unit in a university hospital, and the remaining examinations in week 18-20 in four different county hospitals. The time periods were also different, since the study period was prolonged in Linköping to get a sufficient number of examinations to allow for a comparison. There could be differences in the level of expertise of the operators between the five units that participated in the study and it cannot be excluded that this could have biased the results. Differences in the quality of the ultrasound systems used at the five units could have influenced detection rates due to different quality of the resolution of the image. In the present organization when two screening ultrasounds are offered to pregnant women at all units in the Southeast region other study designs would be possible. A randomized controlled trial is considered to be the most reliable form of scientific study, and could be used for a comparison of first and second trimester ultrasound.

When analyzing the findings over BMI-strata, the number of anomalies in each BMI-group became too small to reveal any differences to be statistically significant. The power calculation yielded a total sample size of 20,250. However the detection rate of anomalies was surprisingly low, which in combination with the low prevalence of obesity in the study population caused the group of obese women to be too small. In

fact, it was impossible to calculate the differences over six BMI strata (with the three obesity classes separate) as planned from the beginning.

A strength of paper III is the combination of qualitative and quantitative analysis used. The qualitative methods used were content analyses of participants “think aloud” notes/protocols during a pre- and post-course test, an audio-recorded group discussion session, and the recorded interviews about using the new technique, including the self-assessment of the performance to assess the fetal heart in clinical practice. One possible improvement might have been to video record the pre-and post-course test for later analysis. Watching the recordings together with the midwives would have given them the opportunity to comment on their own way to think during the test. This might have provided us with more data for analysis. The amount of data from the interviews was also limited. A specialist in interviewing technique might have provided more data. A deeper analysis was thus not possible. Therefore a simple content analysis was performed. The quantitative methods in the study were assessment of the ultrasound recordings by specialists including number of complete recordings and the time used for obtaining the images. Only four midwives participated, and even though they were chosen to represent short and long experience, this might have affected the result. The preferred method to evaluate an improvement in the screening would have been to measure the detection rate of CHD cases after implementation of the new method in clinical use. However the power calculation indicated that this would require an extremely large number of examinations, so large that it would not be possible to collect the necessary data in the region in a reasonable number of years.

When studying the risk-assessment in the combined screening for aneuploidy, data from the ultrasound database Astraia® were used and matched with data from the obstetrical medical record database Obstetrix®. The Astraia database includes risk-assessment for trisomy 13, 18 and 21. We studied the performance of the algorithm in assessing the risk of Down syndrome, and even though other trisomies are rare, it would have been possible to have included them in the analysis.

To find out if there is an association between Down syndrome births and obesity the Swedish Medical Birth register was used due to the large amount of data in the register and the high reliability. During the study period of 16 years more than 1.5 million births with known BMI could be included. Data on socioeconomic status (SES) would have been interesting to include in the analysis, but the register lacks information about SES. Obesity is linked to low SES, and there is a possibility that obese women attend prenatal care to a lesser extent than normal-weight women

(James 1997; Park 2011). The SES might also affect either the woman's acceptance of an offer of prenatal screening or invasive testing in case of an increased risk of aneuploidy. There is also evidence of an association between definitive prenatal diagnosis of Down syndrome and termination rates depending on maternal age, gestational age and maternal ethnicity (Natoli 2012). There could have been benefits of an inclusion of additional factors in the analysis.

DISCUSSION OF THE RESULTS

In the southeast region of Sweden, we noted a slightly lower detection rate of fetal structural anomalies than has been reported in some other studies, but lethal anomalies were detected at a high level. Interestingly, the difference between the 11-14 week scan group and the scan performed in the second trimester in finding lethal anomalies and anomalies associated with high morbidity was not significant. A Norwegian study of screening in the second trimester also in a rural setting had a sensitivity of 39% for detecting major anomalies, 80% for lethal anomalies and 67% for anomalies associated with long-term morbidity (Nakling 2005). The low detection rate of cardiac anomalies was similar to our findings.

Maternal obesity is associated with less complete anatomical surveys, increased number of scans required, decreased detection of fetal anomalies and limitation of the visualization of the fetal organs (Hendler 2004; Hendler 2004; Hendler 2005; Dashe 2009; Thornburg 2009; Aagaard-Tillery 2010; Maxwell 2010). The findings that are of importance for the parents are, of course, those indicating the presence of lethal anomalies and even other anomalies that have the potential for causing future severe handicap in the child. With this approach in mind, we grouped the findings in this large dataset of consecutive routine clinical ultrasound examinations performed in a rural setting in the southeast region of Sweden according to their likely clinical consequences. In absolute numbers it seems as if maternal obesity decreases the ability of detecting anomalies with long-term handicap, although the interpretation of the data must be done with caution because of the small number of detected anomalies. No differences between obese women and normal weight women were found in the group with lethal anomalies but the small number of cases with this condition probably makes it impossible to draw any conclusions. The findings are in accordance with other studies (Dashe 2009; Aagaard-Tillery 2010). The reason for the lower detection rate is probably related to impaired visualization of the fetal structures due to the large amount of subcutaneous fat. Hendler et al. studied the rate of ultrasound with suboptimal visualization, and found that the rate was increased in the obese women (Hendler 2004). In a Swedish study the estimated

gestational age in obese women was found to be shorter according to ultrasound than according to last menstrual period. A possible explanation could be the impairment of the visualization in combination with poorer lateral resolution making the estimated day of delivery incorrect (Simic 2010).

Congenital heart disease (CHD) is a potentially life-threatening condition if not identified before birth or shortly after birth (Hoffman 2002; Tegnander 2006). It is also the most common congenital defect, and just under 1% of all pregnancies are affected by CHD (Mitchell 1971; Cedergren 2003). Data indicate that the prenatal detection rate of CHD in Sweden has a potential for being significantly improved, since the majority of CHD cases remain unidentified during pregnancy (Del Bianco 2006). Training programs in assessing the fetal heart have been effective, and antenatal detection of CHD could be increased significantly by adding the three-vessel view (Carvalho 2002). Color-Doppler added is also a method that has a potential to improve the screening (Eggebo 2012). To explore the potential benefits of a learning program, a two-day course was given in assessing the fetal heart in a more accurate way. The course was followed up by assessment of the images produced in the every-day screening situation. Additionally, the sonographing midwives did a self-assessed documentation of the possibility to perform complete examinations using the new method. At follow up after two years the ability to perform the different steps of the screening remained at the same level. There was a significant difference in the time spent to achieve the projections with advantage to the follow up, and also the ability to perform the color Doppler images had improved after two years in praxis. It seems as if it is possible to improve the skills of the midwives in assessing the fetal heart, but the results indicating this have to be evaluated by including a larger number of midwives and a long-term follow up on the detection rate and potential benefits for the children with CHD.

Screening for fetal aneuploidy is a well-established routine using the combined method including measurement of the nuchal translucency thickness (NT) and combining the results in an algorithm including maternal age, characteristics and biochemistry (Kagan 2009). The biochemistry markers used are s-hCG and PAPP-A from the placenta. In the obese women, there is not only the effect on visualization discussed above that must be kept in mind, but also the dilution-effect on the biomarkers with increased maternal weight or BMI (Spencer 2003). NT has been found to be thicker in obese women, but the effect on the risk-assessment is too small to have an effect in clinical praxis (Cowans 2011). Our aim was to investigate if the risk-assessment was different in the obese women than in the normal-weight women. The correction of weight was found to be sufficient also for BMI concerning

PAPP-A, whereas the correction for hCG was insufficient. However, when scrutinizing this further, the effects of BMI on NT, hCG and PAPP-A did not affect the estimation of risk of Down syndrome beyond critical level, and thus offering an amniocentesis due to a false estimation. This is in accordance with earlier studies (Krantz 2005; Sahota 2009; Rode 2011).

Maternal obesity is known to be associated with birth defects in offspring. There is evidence of an increased risk for neural tube defects, CHD and orofacial clefts (Rasmussen 2008; Stothard 2009; Blomberg 2010; Mills 2010; Rankin 2010). There is, however, no certain known association between maternal pre-pregnancy obesity and the prevalence of Down syndrome in offspring. Down syndrome or trisomy 21 is a result from a non-disjunction in gametogenesis, either in maternal meiosis I or II (Oliver 2012). Little is known about the etiology of trisomy 21, other than the well-known age effect. To investigate this 1,568,604 women who had given birth in Sweden from January 1, 1995 through December 31, 2010 were identified using the Swedish Medical Birth Register (National Board 2003). The age-adjusted risk of giving birth to an infant with Down syndrome was found to be 28% higher in obese mothers compared to those with normal BMI. A possible explanation of the observed association between maternal obesity and Down syndrome could be linked to the association between maternal obesity and the increased risk of NTD. Barkai et al. found evidence of an association between NTD and Down syndrome with a high risk of Down syndrome among families with a high risk of NTD and vice versa (Barkai 2003). This could be due to impaired folate status as supplement with folic acid and iron appeared to have a protective effect against Down syndrome during the first month of pregnancy. However, this hypothesis has been questioned. A recent large study found little evidence for this – only at a maternal age ≥ 35 and a meiosis II error was an association seen but this could be the result of multiple testing (Oliver 2012). The possible link between obesity and aneuploidy needs to be investigated further, also with the aim to find the etiology to Down syndrome.

CONCLUSIONS

- The detection rate of fetal structural abnormalities was significantly higher in the second trimester in comparison to the 11-14 week scan.
- A high percentage of lethal anomalies and anomalies that could lead to future handicap were discovered at both examinations.
- There is a need to improve the detection of cardiac anomalies.
- Screening for fetal anomalies was associated with a lower detection rate in obese women.
- Maternal obesity was associated with a decreased possibility to detect anomalies with long term handicap.
- By implementing new imaging modalities and providing hands-on-training in assessing the fetal heart, uncertainty of the findings can be reduced and time spent on the investigation be decreased.
- Continuous training in assessing the fetal heart with clinical and technical back-up is important.
- The combined screening with ultrasound and biochemistry seemed equally effective irrespective of maternal BMI.
- The risk of giving birth to an infant with Down syndrome was higher among obese women compared to normal weight woman.

FUTURE PERSPECTIVE OF CLINICAL IMPLICATIONS

Following the evolution of high-resolution ultrasound equipment it is not only the possibility to detect a larger proportion of fetal anomalies that makes the screening more effective, but also the opportunity to perform the screening earlier in pregnancy, which in turn poses a new challenge.

At present, the combined screening in 11-14 weeks of gestation, offers an effective method to do a risk assessment of carrying a fetus with Down syndrome or other trisomies. Most probably, the parents-to-be will also expect assessment of the anatomy of the fetus. It is possible to detect about 50% or more major malformations at 11-14 weeks of gestation (Rossi 2013). Further on, the majority of potentially lethal malformations like acrania, lethal skeletal dysplasia, megacystis, encephalocele and body stalk anomaly can be detected in the 11-14 week scan (Hildebrand 2010).

However the factors affecting the effectiveness of the screening, especially maternal obesity require that special strategies be developed or employed. It is possible that prevention of some fetal anomalies could be made with medical supplements, for example folic acid in a sufficient dose. If it is not possible to do a full examination due to impaired visualization additional assessment with vaginal ultrasound is one possibility.

Overall, there are more potential benefits from an improved prenatal screening. If adding information on maternal characteristics and obstetric history, measurement of the cervix and blood flow in the uterine artery a full risk assessment of the pregnancy and outcome is possible. This has important implications for the maternity health care system, where low-risk pregnancies are separated from pregnancies with high risk of fetal structural abnormalities, chromosomal anomalies, preterm labor, intrauterine growth retardation and pre-eclampsia.

SUMMARY IN SWEDISH – SAMMANFATTNING PÅ SVENSKA

ULTRALJUDSUNDERSÖKNING I SYDÖSTRA SJUKVÅRDSREGIONEN

Screeningultraljud under graviditet är en väl etablerad metod i Sverige. Alla gravida i sydöstra sjukvårdsregionen (Landstingen i Östergötland, Jönköping och Kalmar) erbjuds ultraljud som en del av mödrahälsovårdens basprogram. Tidigare gjordes ultraljudet kring graviditetsvecka 18-20 förutom på en enhet i regionen där ultraljudet gjordes i v 11-14. Sedan 2009 erbjuds två ultraljud till alla gravida i hela regionen. Ett första ultraljud görs i graviditetsvecka 11-14 för beräkning av graviditetslängd och för att kunna upptäcka flerbörd. Om kvinnan önskar görs också en uppskattning av risken att bära på ett foster med kromosomavvikelse. Bedömning av organen i form av missbildningsscreening görs i graviditetsvecka 18-20. De flesta ultraljud utförs av barnmorskor med speciell utbildning i ultraljudsdiagnostik.

SCREENING FÖR MISSBILDNINGAR

Frekvensen missbildningar är kring 4% varav hälften är allvarliga. Förmågan att upptäcka missbildningar med ultraljud varierar kraftigt mellan olika studier. Även när det gäller olika organ hos fostret varierar det kraftigt i hur hög grad man kan upptäcka missbildningar. Det är lättast att hitta missbildningar i centrala nervsystemet och urinvägarna, medan hjärtat och skelettet hör till de mest svårundersökta delarna av fostret. Hjärtmissbildningar är bland de vanligaste missbildningarna, och samtidigt alltså svårast att undersöka. De är viktiga att upptäcka före födseln eftersom många kräver tidig operation eller medicinering av barnet, och livshotande tillstånd kan uppstå vid ett okänt hjärtfel.

SCREENING FÖR DOWNS SYNDROM

Tidigare erbjöds screening för Downs syndrom endast till kvinnor som var 35 år och äldre vid konceptionen. Med den metoden kunde 30% av foster med Downs syndrom upptäckas om alla tackade ja till fostervattenprov. Eftersom andelen gravida kvinnor över 35 år ökar, utsätts fler för risken för missfall om man gör ett fostervattenprov. Om man istället gör kombinerat ultraljud och biokemiskt test (KUB) kan >90% av foster med Downs syndrom hittas om man gör fostervattenprov på 4-5% av de gravida kvinnorna. Man mäter då en vätskespalt (nackupplärning, NUPP) i fostrets nacke, och tillsammans med uppgifter om mamman och blodprovresultatet ger det

en sannolikhet att bära på ett foster med Downs syndrom. Om risken bedöms som hög erbjuds ett fostervattenprov.

FAKTORER SOM PÅVERKAR SCREENINGEN

Ultraljudsutrustningen blir allt bättre och ger förbättrade möjligheter att bedöma fostret. Förekomsten av fetma i den gravida populationen ökar samtidigt. Studier har visat att ultraljudsundersökningen tar längre tid och det är svårare att göra den fullständig hos feta. Det är troligen också svårare att hitta missbildningar, men detta är inte studerat i fler än några få studier.

SYFTE MED STUDIEN

- Att undersöka hur stor andel av missbildningarna och kromosomavvikelserna som kan upptäckas vid rutinultraljudet i vecka 18-20 och jämföra resultatet med ultraljudsundersökning i vecka 11-14.
- Att undersöka hur fetma hos den gravida kvinnan påverkar förmågan att upptäcka missbildningar hos fostret.
- Att utvärdera möjligheten att införa en mer fullständig undersökning av fosterhjärtat baserat på fem tvärsnitt av fosterhjärtat och färgdoppler genom att använda ett nytt sätt att träna undersökningen på och dessutom utvärdera denna.
- Att undersöka om fetma ger ökad förekomst av Downs syndrom och om riskberäkningsprogrammet med algoritmen från Fetal Medicine Foundation i London varierar beroende på BMI vid screening för Downs syndrom.

METODER

Analys gjordes av 21189 undersökningar under graviditeten. 6692 är gjorda i vecka 11-14 och 14497 i vecka 18-20. För 19140 undersökningar finns uppgifter om BMI. Data från undersökningen jämfördes med utfallet efter förlossningen genom matchning av ultraljudsfynden med barnundersökningen efter födseln. Om abort eller missfall varit fallet undersöktes även fynden där. Därefter genomfördes en tvådagars intensivkurs i undersökning av fosterhjärtat där färgdoppler och undersökningsteknik lärdes ut till 4 barnmorskor. Utvärdering av kursen skedde genom inspelade grupputvärderingar, självskattning av undersökningsresultat efter kursen i kliniska vardagen och granskning av inspelat material från undersökningar i kliniska vardagen efter kursen. För att se om fetma har en påverkan på riskberäkningen vid KUB samt nyfödda barn granskas två studiepopulationer. Population I består av 1568604 födda barn mellan 1 januari 1995 och 31 december

2010 och population II 10224 kvinnor som genomgår KUB Östergötland mellan 1 april 2009 och 31 december 2011. Population I analyseras avseende BMI och Downs syndrom, och population II avseende sannolikhetsberäkningen att bära på ett foster med Downs syndrom i förhållande till mammans BMI.

RESULTAT

Detektion av missbildningar och kromosomavvikelser

Det var signifikant högre sensitivitet (29%) för att upptäcka missbildningar hos foster i andra trimestern (v 18-20) jämfört med första trimestern (13%) (v 11-14). Det var ingen signifikant skillnad mellan de olika tidpunkterna i graviditeten att upptäcka missbildningar som inte var förenliga med liv (92% respektive 88%) och de som kan orsaka handikapp i framtiden (44% respektive 35%). En större andel av kromosomavvikelserna upptäcktes i första trimestern jämfört med andra (71% respektive 42%)

Påverkan på upptäckt av missbildningar på grund av högt BMI

Hos underviktiga kvinnor upptäcktes 20% av missbildningarna, hos normalviktiga 26%, överviktiga 29% och feta 19%. I absoluta tal verkar det som om fetma minskar möjligheten att upptäcka missbildningar hos foster, men siffrorna ska tolkas med försiktighet på grund av att det är få fall inom varje viktgrupp.

Utvärdering av möjligheten att införa en mer fullständig undersökning av fosterhjärtat baserat på fem tvärsnitt av fosterhjärtat och färgdoppler genom att använda ett nytt sätt att träna undersökningen på och dessutom utvärdera denna.

Det verkar möjligt för barnmorskor att få ökad kompetens att bedöma fosterhjärtat på ett korrekt sätt genom ett välstrukturerat träningsprogram och på det sättet öka möjligheterna att upptäcka hjärtmissbildningar hos foster vid ultraljudsundersökningen.

Association mellan fetma och Downs syndrom hos födda barn och variationen av sannolikhetsbedömningen vid KUB beroende på BMI.

Risken att föda ett barn med Downs syndrom var högre hos feta kvinnor jämfört med normalviktiga efter justering för ålder hos kvinnan. Riskbedömningen med KUB verkar lika effektiv i alla BMI-grupper.

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ERRATA

Article	Journal/page/line	Original text	Corrected text
Comparison of first and second trimester ultrasound screening for fetal anomalies in the southeast region of Sweden	Acta Obstetrica et Gynecologica Scandinavica, 89: 1412-1419, page 1414/Results/line 24	Excluding minor anomalies, the prevalence of fetuses and babies with at least moderate anomalies was 2% (408/21,189)	Excluding minor anomalies, the prevalence of fetuses and babies with at least moderate anomalies was 2% (421 /21,189)
Maternal obesity and risk of Downs syndrome in the offspring	Prenatal diagnosis 2013,33,1-6, page 1/Abstract/Methods line 1 and page 2/Methods/line 1.	168 604 women	1 568 604 women

Papers

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