HEALTH SCIENCES

Jenni Ranta-Ylikulju

First Trimester Down´s Syndrome Screening

The Role of Biochemistry

Publications of the University of Eastern Finland
Dissertations in Health Sciences

UNIVERSITY OF
EASTERN FINLAND

JENNI RANTA-YLIKULJU

First trimester Down's syndrome screening – the role of biochemistry

To be presented by permission of the Faculty of Health Sciences, University of Eastern Finland for public examination in Mediteknia Auditorium, Kuopio, on Friday May 11th 2012, at 12 noon

Publications of the University of Eastern Finland
Dissertations in Health Sciences
Number 111

Department of Clinical Chemistry and Department of Obstetrics and Gynaegolocy,
Institute of Clinical Medicine, School of Medicine,
Faculty of Health Sciences,
University of Eastern Finland
Kuopio
2012

Kopijyvä Ltd.
Kuopio, 2012

Distributor:
University of Eastern Finland
Kuopio Campus Library
P.O.Box 1627
FI-70211 Kuopio, Finland
http://www.uef.fi/kirjasto

ISBN (print): 978-952-61-0769-1
ISBN (pdf): 978-952-61-0770-7
ISSN (print): 1798-5706
ISSN (pdf): 1798-5714
ISSN-L: 1798-5706

Author's address: Department of Clinical Chemistry, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
KUOPIO
FINLAND

Supervisors: Professor Kari Pulkki, M.D., Ph.D.
Department of Clinical Chemistry, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
KUOPIO
FINLAND

Jarkko Romppanen M.D., Ph.D.
Eastern Finland Laboratory Centre
Department of Clinical Chemistry, Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
KUOPIO
FINLAND

Professor Seppo Heinonen M.D., Ph.D.
Department of Obstetrics and Gynaegolocy,
Institute of Clinical Medicine
School of Medicine, Faculty of Health Sciences
University of Eastern Finland
KUOPIO
FINLAND

Reviewers: Professor Pertti Kirkinen, M.D., Ph.D.
Department of Obstetrics and Gynaegolocy
University of Tampere
TAMPERE
FINLAND

Professor Onni Niemelä M.D., Ph.D.
Department of Laboratory Medicine
University of Tampere
TAMPERE
FINLAND

Opponent: Professor Ulf-Håkan Stenman, M.D., Ph.D.
Department of Clinical Chemistry
University of Helsinki
HELSINKI
FINLAND

Ranta-Ylikulju, Jenni Katriina
First trimester Down's syndrome screening – the role of biochemistry, 53 p.
University of Eastern Finland, Faculty of Health Sciences, 2012
Publications of the University of Eastern Finland. Dissertations in Health Sciences 111. 2012. 53 p.

ISBN (print): 978-952-61-0769-1
ISBN (pdf): 978-952-61-0770-7
ISSN (print): 1798-5706
ISSN (pdf): 1798-5714
ISSN-L: 1798-5706

ABSTRACT

Maternal and foetal characteristics affect the concentrations of markers used in first trimester screening (FTS). Subfertility, manifested as a prolonged waiting time to pregnancy (TTP), has been associated with adverse pregnancy outcomes. It has been reported that the levels of free beta human chorionic gonadotropin (fβ-hCG) and pregnancy associated plasma protein A (PAPP-A) in the maternal serum deviate from the norm in adverse outcomes such as preterm delivery (PD), preeclampsia (PE), and small for gestational age (SGA) infants. In addition, the performance of the combined first trimester screening (FTS) is known to be superior in older women.

The study focused on characteristics affecting the quality of FTS. The screening markers for Down's syndrome (DS) in spontaneous, chromosomally normal pregnancies with different TTPs were compared to the results of those for pregnancies initiated using *in vitro fertilization* (IVF). Adverse pregnancy outcomes – PD, PE, SGA, and placental abruption (PA) – and their DS screening results were examined. In a multicenter study, the detection rates and the number of invasive procedures needed to detect a single case of Down's syndrome were analyzed at different maternal ages.

The main finding was that PAPP-A, which is monitored as part of the screening protocol for DS, is the best marker for subfertility and adverse pregnancy outcomes, and that its concentration in younger women is significantly lower than is the case in older women. The median multiple of median (MOM) values for PAPP-A concentrations in spontaneous pregnancies with a TTP over two years and in IVF pregnancies were significantly lower than those observed for pregnancies with a TTP of less than one year. Because the PAPP-A levels for spontaneous pregnancies with TTPs in excess of two years were comparable to those for IVF pregnancies, it seems that low PAPP-A concentrations may be related to subfertility rather than the use of artificial reproductive technology. The median MOM for PAPP-A was significantly lower in pregnancies with PD, PE or SGA, but no difference was found in pregnancies with PA. The lower the concentrations of PAPP-A and fβ-hCG, the higher the odds ratios for developing PD or SGA. The mechanism behind PA is probably less dependent on the placenta than the decidua, as no difference in markers of the FTS was seen. In DS pregnancies, the detection rate when using the biochemical markers alone was significantly higher for women who were ≥35 years than for younger women. Therefore, for younger women, combined screening should be the method of choice. For a fixed false positive rate of five percent, the number of invasive procedures needed to detect one case of DS would be higher in younger than in older women.

National Library of Medical Classification: WS 107, WQ 209, WQ 210.5, WH 400, QY 455

Medical Subject Headings: Down Syndrome/diagnosis; Pregnancy Trimester, First; Biological Markers; Pregnancy-Associated Plasma Protein-A; Pregnancy Outcome; Prenatal Diagnosis

Ranta-Ylikulju, Jenni Katriina
Ensimmäisen raskauskolmanneksen Downin oireyhtymän seulonta – biokemiallisten merkkiaineiden rooli, 53 s.
Itä-Suomen yliopisto, Terveystieteiden tiedekunta, 2012.
Publications of the University of Eastern Finland. Dissertations in Health Sciences 111. 2012. 53 s.

ISBN (print): 978-952-61-0769-1
ISBN (pdf): 978-952-61-0770-7
ISSN (print): 1798-5706
ISSN (pdf): 1798-5714
ISSN-L: 1798-5706

TIIVISTELMÄ

Odottavan äidin, istukan ja sikiön ominaisuudet vaikuttavat ensimmäisen raskauskolmanneksen Downin oireyhtymän seulonnan merkkiaineisiin. Seulonnan tiedetään havaitsevan paremmin vanhempien kuin nuorempien naisten Down-raskauksia. Alentunut hedelmällisuus, joka ilmenee pidentyneenä raskauden alkamisen viiveenä, on liitetty raskauden poikkeaviin lopputuloksiin, kuten pre-eklampsiaan eli raskausmyrkytykseen, sikiön kasvuhäiriöön ja ennenaikaiseen synnytykseen. Ensimmäisen raskauskolmanneksen seulonnan biokemiallisten merkkiaineiden – istukkahormonin vapaan beeta-alayksikön (vapaa β-hCG) ja raskaudenaikaisen plasmaproteiini A:n (PAPP-A) pitoisuudet on todettu epänormaaleiksi näissä komplisoiduissa raskauksissa.

Väitöskirjatutkimuksessa tarkasteltiin seulonnan laatuun vaikuttavia tekijöitä. Raskausviiveen vaikutusta merkkiaineisiin verrattiin kromosomeiltaan normaaleissa spontaanialkuisissa ja keinoalkuisissa raskauksissa. Poikkeavien raskauden lopputulosten – myös istukan ennenaikaisen irtoamisen – merkkiaineiden pitoisuuksia verrattiin normaaliraskauksiin. Lisäksi seulonnan havaitsemisasteita ja tarvittavien kromosomitutkimusten määriä yhden Down-raskauden havaitsemiseksi vertailtiin odottavien äitien eri ikäryhmien välillä.

PAPP-A:n todettiin olevan paras yksittäinen merkkiaine Down-raskauksien lisäksi raskausmyrkytykselle, ennenaikaiselle synnytykselle tai sikiön kasvuhäiriölle. Mitä matalammat vapaan β-hCG:n ja PAPP-A:n pitosuudet, sitä korkeampi riskisuhde ennenaikaiselle synnytykselle ja ikäänsä nähden pienipainoiselle sikiölle. Istukan ennenaikaista irtoamista ei voitu ennustaa merkkiaineista. PAPP-A:n pitoisuus oli matala myös keinoalkuisissa raskauksissa sekä spontaanialkuisissa raskauksissa, joissa oli yli kahden vuoden alkamisviive, verrattuna raskauksiin, joissa alkamisviive oli enintään yhden vuoden. Koska raskaaksi tulemisen viive laskee PAPP-A:n pitoisuutta hedelmöityshoitojen tavoin, voi PAPP-A:n lasku liittyä alentuneeseen hedelmällisyyteen ennemmin kuin keinoalkuiseen raskauteen. Seerumiseulonta toimii parhaiten vanhemmilla naisilla. Nuoremmilla naisilla yhdistelmäseulonta on ensisijainen menetelmä. Down-raskauksissa nuorille, <35-vuotiaille, naisille tarvittiin enemmän kromosomitutkimuksia Down-raskauksien löytämiseksi ja havaitsemisaste seerumiseulonnassa oli merkittävästi huonompi kuin vanhemmilla, ≥35-vuotiailla, naisilla.

Luokitus: WS 107, WQ 209, WQ 210.5, WH 400, QY 455
Yleinen Suomalainen asiasanasto: Downin oireyhtymä - - diagnoosi; sikiödiagnostiikka; raskaus; ensimmäinen raskauskolmannes; raskauteen liittyvä plasman proteiini A; seulonta

Acknowledgements

The studies reported herein were conducted in the Eastern Finland Laboratory Centre and the Department of Obstetrics and Gynaecology of Kuopio University Hospital during the years 2007–2012. I owe sincere thanks to my supervisors, Professor Kari Pulkki (M.D., Ph.D.) and Professor Seppo Heinonen (M.D., Ph.D.) who have always provided me with their guidance and given me inspiration, and encouragement when I needed it the most, as well as Assistant Senior Physician Jarkko Romppanen (M.D., Ph.D.), who has guided me concerning the practicalities involving research and in using different tools in data collection. The most valuable valuable lesson I have learned from you three is to strive for good quality in research and in the subject of the research, screening. I thank you for introducing me to scientific research.

I am grateful to Professor Markku Ryynänen (M.D., Ph.D.) in the Department of Obstetrics and Gynaecology of Oulu University Hospital and Docent Päivi Laitinen (Ph.D) in Helsinki Central University Hospital Laboratory for your supervision and guidance of my research. You have always been supportive and working with you has always been pleasant. I thank my colleague researcher Jaana Marttala (B.M., dissertation) in the Department of Obstetrics and Gynaecology of Oulu University Hospital for sharing her experiences and support when working with me in the field of first trimester screening.

I want to thank the staff at the Eastern Finland Laboratory Centre's Department of Clinical Chemistry, especially Tero Hongisto (B.L.S.) for always being so helpful, as well as for his friendship. I am also grateful to specialist of clinical chemistry Susanna Luukkonen (M.D.) for her supervision and guidance of my advanced studies in medicine, which inspired me to take on this doctoral project. In the Department of Clinical Genetics, I wish to thank Senior Physician, Docent Tarja Mononen (M.D., Ph.D.) and Nurse of Ward Helena Swahn for allowing me access to the register of chromosomes, which was crucial for this study. I also thank biologists Raija-Liisa Airaksinen (M.Sc.) and Kristiina Heinonen (M.Sc.) for providing me with photographs presented in this thesis. In the Department of Clinical Microbiology, I am grateful to Professor Jukka Pelkonen (M.D., Ph.D.), for conversations on this research and guidance in grant application.

In the Department of Obstetrics and Gynaecology of Kuopio University Hospital, I owe my sincere thanks to gynaecologist Kaisa Raatikainen (M.D., Ph.D.) for her significant guidance in writing my very first scientific article, Docent Leea Keski-Nisula (M.D., Ph.D.) for advice on writing my study plan, Assistant Senior physician Maija-Riitta Ordén (M.D., Ph.D.) for providing me with a photograph presented in this thesis, as well as Olavi Kauhanen (M.Sc.) for his expertise in statistics and being professional pleasant to work with.

I want to thank Docent, Senior Physician Esa Hämäläinen (M.D., Ph.D.) in Women's Clinic Laboratory, Helsinki Central University Hospital and the Department of Clinical Chemistry in University of Helsinki for providing the data concerning screening, and assessment of a research article. I also wish to thank Senior Physician Jaana Fraser (M.D.) and Assistant Senior Physician Rauno Solja (M.D.) in Gynaecology and Obstetrics of North Karelia Central Hospital, Joensuu; Senior Physician Marja-Liisa Mäntymaa (M.D.) in Gynaecology and Obstetrics of Kymenlaakso Central Hospital, Kotka; Senior Physician Helena Sundström (M.D., Ph.D.) and Senior Ward Physician Jaana Kröger (M.D.) in Gynaecology and Obstetrics of Central Finland Central Hospital, Jyväskylä; gynaecologist Anna-Maria

Parviainen (M.D.) in Central Finland Central Hospital and Gyne-Paxis Ltd, Jyväskylä; Senior Physician Timo Tiilikainen in Obstetrics and Gynaecology of Mikkeli Central Hospital; and Senior Physician Martti Ämmälä (M.D., Ph.D.) in Gynaecology and Obstetrics of Hyvinkää Hospital for their important aid in data collection for this study.

I am grateful for Professor Pertti Kirkinen (M.D., Ph.D.) and Professor Onni Niemelä (M.D., Ph.D.) for their valuable contribution in reviewing my thesis. You have provided me with observations that have substantially improved the quality of the final work.

I want to express my gratitude to my superior Outi J. Nyberg (M.D.) in the Länsi-Pohja Central Hospital, Kemi for giving me time from work to finish this thesis.

My loving thanks go to my husband Mikko Ylikulju for his understanding towards the requirement of time this process has demanded, and always showing his support bringing me back to light when I have felt lassitude. I thank dearly my mother Kaisu Rahko, for being a paragon of a woman of science for me since I was a little girl, as well as my father Rauno Ranta for his endless love and care. I thank my brothers Juuso and Jani, along with his family, for their caring and friendship. I thank my dear friends for taking care of my mental health during this process.

The study was financially supported by The Faculty of Health Sciences in the University of Eastern Finland, Kuopion yliopistosäätiö, and Laboratoriolääketieteen Edistämissäätiö and Suomen kliinisen kemian erikoislääkäriyhdistys.

Jenni Ranta-Ylikulju
Oulu, April 2012

List of the original publications

This dissertation is based on the following original publications:

I Increased time-to-pregnancy and first trimester Down's syndrome screening.
Ranta JK, Raatikainen K, Romppanen J, Pulkki K, Heinonen S.
Hum Reprod. 2010 Feb;25(2):412-7. Epub 2009 Nov 26.

II Decreased PAPP-A is associated with preeclampsia, premature delivery and small for gestational age infants but not with placental abruption.
Ranta JK, Raatikainen K, Romppanen J, Pulkki K, Heinonen S.
Eur J Obstet Gynecol Reprod Biol. 2011 Jul;157(1):48-52. Epub 2011 Apr 8.

III More invasive procedures are done to detect each case of Down's syndrome in younger women.
Marttala J, Ranta JK, Kaijomaa M, Nieminen P, Laitinen P, Kokkonen H, Romppanen J, Hamalainen E, Kultti J, Tekay A, Ulander VM, Honkasalo T, Ryynanen M.
Acta Obstet Gynecol Scand. 2011 Jun;90(6):642-7. doi: 10.1111/j.1600-0412.2011.01113.x. Epub 2011 Apr 15.

IV First trimester biochemistry at different maternal ages.
Ranta JK, Marttala J, Laitinen P, Kultti J, Kauhanen O, Romppanen J, Hämäläinen E, Heinonen S, Pulkki K, Ryynänen M.
Clin Chem Lab Med. 2011 Nov 24. Volume 50, Issue 3, Pages 549–555, ISSN (Online) 1437-4331, ISSN (Print) 1434-6621, DOI: 10.1515/cclm.2011.785. 2011 Nov 24. [Epub ahead of print]

Contents

Abbreviations

ADAM12	A disintegrin and metalloproteinase 12
AFS	Amniotic fluid sample
ART	Assisted reproductive technology
BMI	Body mass index
CRL	Crown-rump length
CVS	Chorionic villus sample
DR	Detection rate
DS	Down's syndrome
fβ-hCG	Free beta subunit of human chorionic gonadotrophin
FinOHTA	the Finnish Office for Health Technology Assessment
FPR	False positive rate
FTS	First trimester screening
IGFBP	Insulin-like growth factor binding protein protease
IGF	Insulin-like growth factor
IUGR	Intrauterine growth restriction
IVF	In vitro fertilisation
LBW	Low birth weight
MOM	Multiple of a median
NT	Nuchal translucency
PA	Placental abruption
PAPP-A	Pregnancy associated plasma protein A
PD	Preterm delivery
PE	Preeclampsia
SGA	Small for gestational age
STAKES	The Centre for Research and Development of Welfare and Health (Sosiaali- ja terveysalan tutkimus- ja kehittämiskeskus
THL	The Finnish National Institute for Health and Welfare (Terveyden ja hyvinvoinnin laitos)
TTP	Time to pregnancy
uE3	Unconjugated estriol
WHO	World Health Organization

1 Introduction

Routine first trimester screening for Down's syndrome is based on knowledge of maternal age together with measuring the levels of biochemical markers produced by the placenta, and ultrasonic measurements of foetal nuchal translucency (NT). Two maternal serum biochemical markers, free β-human chorionic gonadotropin (fβ-hCG) and pregnancy-associated plasma protein-A (PAPP-A), are used to determine whether the placenta is maturing more slowly than would be expected considering the gestational age. Although the first trimester screening (FTS) focuses on Down's syndrome, it could potentially also be used to identify any form of abnormal pregnancy involving an immature placenta, i.e. the other major trisomies (i.e. cases where three copies of a give chromosome are present in a diploid cell) such as trisomy 13 and 18 (Spencer and Nicolaides 2002, Wapner et al. 2003, Breathnach et al. 2007, Kagan et al. 2008), as well as adverse pregnancy outcomes with a normal foetal karyotype, such as preterm delivery (PD), preeclampsia (PE), and small for gestational age (SGA) foetuses (Smith et al. 2002, Tul et al. 2003, Dugoff et al. 2004, Liu et al. 2004, Brameld et al. 2008, Spencer et al. 2008a, Spencer et al. 2008b, Poon et al. 2009). It has been shown that low concentrations of PAPP-A are particularly common in pregnancies with such outcomes. Abnormal fβ-hCG and PAPP-A levels have also been reported in pregnancies due to *in vitro fertilisation* (IVF), although the frequency of trisomy 21 is virtually unaffected by the use of assisted reproduction (Hui et al. 2006, Gjerris et al. 2009).

The studies reported in this thesis focused on different aspects of first trimester Down's syndrome screening, and the characteristics that affect the quality of the results. In normal singleton pregnancies, we studied the association between the waiting time to pregnancy (TTP), measured from the start of attempts to become pregnant to the observation of an actual pregnancy, and the combined FTS markers. In addition, we examined how the markers of the combined screening behave in different adverse outcomes: PD, PE, SGA, and placental abruption (PA). Furthermore, we compared the influence of maternal age on the levels of fβ-hCG and PAPP-A in both chromosomally normal and Down's syndrome pregnancies.

2 Review of literature

2.1 DOWN'S SYNDROME

Down's syndrome (DS) was named after Langdon Down, a doctor from London who first described the condition in 1866 and reported it to be genetic in origin (Down 1866). The prevalence of Down's syndrome and the various mental disabilities it causes in the population is about 0.1 percent (Working group appointed by the Finnish Medical Society Duodecim and The Finnish Association of Doctors in Developmental Disability 2010). DS is known to be the most important cause of mental disability, affecting approximately 10 percent of the mentally disabled population (Hagberg and Kyllerman 1983).

DS is caused by triploidy of chromosome 21 and is therefore also known as trisomy 21. Triploidy normally occurs as a result of incorrect chromosomal segregation during mitosis; in 80–90 percent of all cases, the additional chromosome 21 is maternal and results from nondisjunction, whereby the chromosome pairs fail to separate properly, resulting in one cell with 2 copies of chromosome 21 and a second with none (Mattei et al. 1980, Mikkelsen 1980, Nicolaidis and Petersen 1998, Petersen and Mikkelsen 2000). It is estimated that nondisjunction is typically caused by ovarian damage incurred prior to ovulation. Approximately four percent of DS cases are believed to arise from translocations between chromosomes 21 and 13, 14, or 15. In addition, approximately one percent of DS cases are caused by mosaicism – the presence of at least two genetically different kinds of cells in an individual that originated from the same fertilised egg, the oozygote. Mosaic aneuploidy, i.e. the absence of a specific chromosome or the presence of an extra one in some but not all cells of the body, is relatively common in preimplanted embryos (Bielanska et al. 2002, van Echten-Arends et al. 2011). The extent to which these individuals exhibit the symptoms of DS depends on their degree of mosaicism. Hereditary factors are not considered to play a major role in determining the likelihood that any given pregnancy will result in DS. Instead, it is believed that the likelihood of DS increases with maternal age and is also higher for twin pregnancies (Bliumina et al. 1975, Rodis et al. 1990), in cases where the mother has sustained ovarian damage prior to puberty and menarche, and issues related to folic acid metabolism (Finley 1975, James et al. 1999).

The risk of trisomy 21 increases with increasing maternal age. At week 12 of pregnancy, the risk of DS for a 30-year old woman is estimated to be 1:626. Approximately one third of foetuses with DS are spontaneously aborted in the early stages of pregnancy, and so the risk at birth is approximately 1:800. The equivalent risks in a 40-year old woman are 1:68 and 1:97, respectively (Snijders et al. 1999). In the Finnish population, the risk of recurrent DS pregnancy is estimated to be approximately two percent in women aged <35 years, and approximately eight percent in women aged ≥35 years (Leskinen et al. 1996).

Numerous health problems are associated with DS. Defects in mental abilities, especially relating to development of speech, as well as mental retardation, are common. Individuals with DS are susceptible to infections and leukemia. Anomalies of the digestive tract and cardiovascular system are common, and congenital heart defects occur in 40–50 percent of DS individuals. Atresias – also known as congenital obturations – are also common and often require early surgical repair. In addition, problems with eyesight and hearing are common (Roizen et al. 1993). There are a

characteristic set of facial features associated with DS and affected individuals tend to be shorter than the population average. In addition, the head circumference of DS children is usually smaller than average. Individuals with DS have a tendency to become overweight, and the functioning of their gastrointestinal system is slow. Obstipation and gallstones are both common from childhood onwards (Buchin et al. 1986). The risks of hypothyroidism and diabetes are high (Loudon et al. 1985). Men with DS are almost invariably infertile, but women are not. In addition to somatic problems, comorbidity with psychiatric diseases is common. Dementia usually affects individuals with DS earlier than the rest of the population, often becoming apparent in the early 60's (Lai et al. 1989). Approximately 10 percent of DS individuals are at least somewhat autistic, and depression is twice as common in DS individuals compared to groups with different mental disabilities (Collacott et al. 1992). The life expectancy of individuals with DS is approximately 50–65 years. Despite these difficulties, most people with DS live happy lives, especially if they are given enough support to live independently.

2.2 ADVERSE OUTCOMES IN CHROMOSOMALLY NORMAL PREGNANCIES

Adverse pregnancy outcomes are those that are associated with elevated mortality, a need for extensive prenatal, perinatal and postnatal follow-up, and subsequent needs for intensive care of either or both of the pregnant woman and the neonate. Adverse pregnancy outcomes often have negative effects on the child that persist for many years (Yanney and Marlow 2004, Marlow et al. 2005, Duley 2009). In addition to their impact on quality of life, adverse pregnancy outcomes are associated with increased direct and indirect costs for the health care system (Cuevas et al. 2005, Desgualdo et al. 2011, Bérard et al. 2012).

In this work, we focused on the following major adverse pregnancy outcomes: placental abruption (PA), preterm delivery (PD), preeclampsia (PE), and small for gestational age (SGA) infants. Whilst it is known that chronic maternal diseases such as hypertension increase the risk of an adverse pregnancy outcome (Mavalankar et al. 1992, Catov et al. 2008), the vast majority of such outcomes occur in healthy pregnant women with no known chronic diseases (Toivonen et al. 2002). Other adverse outcomes were excluded, including the effects of alcohol or drug exposure during pregnancy. In Finland, approximately 500 children (0.8 percent of all newborns) that have been adversely affected by alcohol are born each year, and these infants may suffer from problems such as neurological development disorders, impaired general and skeletal growth, and changes in hormone secretion (van Faassen and Niemelä 2011).

2.2.1 Placental abruption

Abruption of the placenta is defined as a partial or complete premature disengagement of the placenta between gestational weeks 22–37. PA occurs in approximately 0.4–1.2 percent of deliveries (Kåregård and Gennser 1986, Saftlas et al. 1991, Ananth and Wilcox 2001) and is a major risk for foetal and neonatal mortality (Oyelese and Ananth 2006, Pariente et al. 2011, Tikkanen 2011). It also endanger the health of the pregnant woman because it causes hypovolemic shock due to massive bleeding and disseminated intravascular coagulopathy. Amniotic embolus may also be a fatal complication. The diagnosis of PA is often clinical in the most severe cases, its onset is very rapid, and PA may cause an obstetric emergency involving bleeding,

hypovolemia, painful and long-lasting contractions of the uterus, foetal asphyxia, and changes in the cardiotocography. In oligosymptomatic cases with less bleeding, ultrasound is used to detect intrauterine bleeding or hematoma. The treatment of PA is based on hypovolemic shock repair, artificial rupture of the membranes in order to reduce the uterine pressure, emergency Caesarean section, treatment of disseminated intravascular coagulopathy by rapid evacuation of the uterine cavity, and replacement of blood products (Montagnana et al. 2010).

The actual abruption is caused by decidual vascular damage. The causes of PA are multifactorial; it is indicated by placental and intrauterine disturbances, such as early and abnormal vascularisation (Dommisse and Tiltman, 1992), inflammation (Steinborn et al. 2004), PE (Wang and Yang 2010), maternal hypertension, excess alcohol consumption during pregnancy (Leunen et al. 2003), intrauterine infection, premature rupture of membranes, polyhydramnion, trauma to the uterus (Melamed et al. 2011), prior uterine curettage, and advanced maternal age. It can also be induced by the birth of the A twin in cases of multiple pregnancy, which causes a sudden decrease in uterine volume. Some familial clustering has also been observed (Toivonen et al. 2004). Despite risk factor mapping, the occurrence of PA is mostly unpredictable, and there is no efficient method for its prevention.

2.2.2 Preterm delivery

PD or preterm birth is defined as birth at any point before the end of the 37th week of pregnancy. According to the Finnish Register of births, 5.9 percent of living newborns were delivered prematurely between 1999 and 2009 (Vuori and Gissler 2011). A World Health Organization (WHO) working group estimated that the percentage of PD in developed countries is five to seven percent, but that it is substantially higher in developing countries, leading to an incidence of 9.6 percent worldwide (Beck et al. 2009). Risk factors include prior PD or having given birth to a low birth weight (LBW) infant, prior foetal intrauterine death, prior induced abortions or miscarriages (Harlap and Davies 1975), chronic maternal illnesses such as hypertension (Czeizel and Bánhidy 2011), multiple pregnancy (Koivisto et al. 1975, Roberts et al. 1990), bleeding during the pregnancy, PA, placenta praevia, PE, intrauterine growth restriction (IUGR) (Horta et al. 1997, Favilli et al. 2011), obstetric cholestasis, social problems during the pregnancy, alcoholism, smoking, primigravida, and very young or very advanced maternal age (Altmann and Kucera 1975, Hiersche et al. 1975). PD is therefore often associated with other major adverse pregnancy outcomes and consequences for SGA newborns. Because PD is multifactorial, it is often impossible to predict its occurrence. It is therefore best treated preventatively, by taking steps to ensure a pregnancy of the normal duration (Saling et al. 2001).

2.2.3 Preeclampsia

A woman is defined as suffering from PE if her blood pressure is measured to be above >140/90 mmHg on multiple occasions, with proteinuria of >0.3 g/day (American Congress of Obstetricians and Gynecologists 2002). According to WHO, PE occurs in approximately three percent of pregnant women in developing countries, and approximately one percent in developed countries (Dolea and AbouZhar 2003). Its onset usually occurs during weeks 32–36 of the pregnancy. PE is considered to be a preliminary stage of eclampsia, and any convulsions it causes are regarded as actual eclamptic seizures. Severe PE can cause swelling, headaches, epigastric pain, and oliguria i.e. reduced diuresis. Approximately 20 percent of women with severe PE develop HELLP (i.e. hemolysis, elevated liver enzymes, low

platelets) syndrome (Sibai et al. 1993), in which the normal coagulation system is disturbed (Weinstein 1982).

The aetiology of PE is currently unknown; vasoconstriction and thrombocyte aggregation are involved (Maynard et al. 2008), and the early placental vascularisation is incomplete. The levels of angiogenic factors such as vascular endothelial growth factor and placental growth factor are reduced (Clark et al. 1998, Lim et al. 2008,), while levels of antiangiogenic factors such as the soluble vascular endothelial growth factor receptor (Clark et al. 1998, Vaisbuch et al. 2011) and the soluble fms-like tyrosine kinase are increased (Lim et al. 2008, Woolcock et al. 2008). Known risk factors include primigravida, multiple pregnancy, maternal obesity, familial clustering (Mandal et al. 2011) and polycystic ovarian syndrome (Kjerulff et al. 2011). By reducing placental blood flow, PE impairs foetal growth, causes asphyxia, and may increase the perinatal death rate (Andersch et al. 1984). Daily treatment with low doses of acetylsalicylic acid starting in the early stages of pregnancy has been reported to reduce the incidence of PE (Bujold et al. 2009, WHO 2011), although this finding is somewhat controversial and only supports the use of aspirin in high-risk populations (Rossi and Mullin 2011, Trivedi 2011). PE screening is performed as a matter of routine in maternity care units, and when diagnosed, the aim of the treatment is to prevent the condition from getting worse by administering medication to reduce blood pressure and imposing bed rest. Patients with PE face an increased risk of cardiovascular diseases later on life (Smith et al. 2001, Pell et al. 2004).

2.2.4 Small for gestational age infants

SGA infants are smaller in size for their sex and gestational age than normal infants, i.e. their sex- and age-adjusted birth weight puts them into the 5th or 10th percentile for all infants or is more than two standard deviations below the mean, depending on the definition being used. Some small infants may be constitutionally small – if the mother is small, her child being small is probably benign. However, if the infant has been subject to IUGR during the pregnancy, they are almost certain to be SGA. SGA indicates that the foetus was unable to achieve its complete potential genetic growth. By definition, the term SGA takes the infant's gestational age into account, whereas LBW is defined in absolute terms as a birth weight below 2500 grams, regardless of the stage of gestation at birth. Approximately 1/3 of infants with LBW are also SGA.

There are both maternal and placental risk factors for SGA; in both cases, they are generally caused by deficiencies in nutrition or circulation. Specific risk factors include famine or malnutrition, excessive physical exertion during pregnancy, alcohol consumption, medication (Ostesen 1994, Nakhai-Pour et al. 2010, Galbally et al. 2011), smoking, and insufficient vitamin D supplementation (Bodnar et al. 2010). Certain medical conditions also present risk factors, including placenta circumvallata, placental hemangiomas, bleeding during pregnancy, multiple pregnancy, foeto-foetal transfusion syndrome, infections during pregnancy, low weight gain during pregnancy, low oxygen supply (which is a consequence of PE), hypertension, vascular collagenous diseases, metabolic diseases (Diderholm 2009), diabetes, and placental infarctions. Finally, certain genetic abnormalities increase the risk of SGA, including trisomies, Turner's syndrome, deletions, placental mosaicism, and other anomalies (Wilkins-Haug et al. 1995). Both IUGR and SGA are screened for during pregnancy by monitoring maternal weight gain and foetal palpation, by measuring the abdominal circumference and the height of the uterus, and by ultrasonography. The treatment of SGA depends on the severity of the situation and

whether the IUGR is increasing. If necessary, the pregnancy is terminated by inducing delivery or Caesarean section, provided that the risks of immaturity are considered less severe than those presented by continuing the pregnancy.

2.3 SCREENING FOR DOWN'S SYNDROME

2.3.1 Preconditions of Down's syndrome screening

According to WHO, screening for a disease or condition should be performed if the following criteria are satisfied: 1) the screened condition should be a major health problem, 2) there should be an acceptable treatment if the condition is diagnosed, as well as 3) facilities for diagnosis and treatment, 4) the condition should have a recognizable latent stage or early symptomatic stage, 5) there should be suitable screening test for the disease that has few false positives (i.e. the test should be specific) and few false negatives (i.e. the test should be sensitive), 6) the screening test should be acceptable to the population, 7) the natural history of the condition should be adequately understood, 8) there should be an agreement regarding which patients should be treated, 9) the cost, including diagnosis and subsequent treatment, should be economically balanced in relation to expenditure on medical care as a whole, and 10) case finding should be a continuous process (WHO 1968).

A report produced by the Finnish Office for Health Technology Assessment (FinOhta) in 2005 discussed the objectives of screening for chromosomal abnormalities, and posed questions that should be considered when offering a screening. Specifically, it asked whether it is justifiable to screen for an abnormality that can not be treated and will often lead to miscarriage, or intrauterine or neonatal death, and if the screening is considered necessary, how big should the risk of the abnormality be in order to consider the screening and invasive testing reasonable in relation to the increased risk of miscarriage caused by the invasive tests (Autti-Rämö et al. 2005).

The different methods used in foetal screening differ from one-another in terms of the gestational age at which they can be applied and the nature of the screened condition. There is no optimal point in time for all conditions to be screened. If the objective of the screening campaign is to identify pregnancies with major chromosomal abnormalities and the foetal chromosomes are to be tested, with the possibility that the pregnancy may be terminated on the basis of the results, the screening must be performed at a sufficiently early stage to enable karyotyping to be completed before it becomes unlawful to perform an abortion (The law on pregnancy abortion 1970, National Supervisory Authority for Health and Welfare 2012).

Because screening of foetal chromosomal abnormalities and anomalies is always fraught with ethical issues, screening should be performed at the earliest possible stage using the most precise method available. This maximises the amount of time available for the pregnant woman and her family to discuss their participation in potentially invasive procedures and the continuation or termination of the pregnancy. When a pregnant woman participates in screening, she must be informed that if invasive testing will be needed to diagnose the condition and to investigate the foetal chromosome, invasive tests carry a risk of miscarriage (Autti-Rämö et al. 2005).

According to our estimates, approximately 70 percent of pregnant Finnish women underwent FTS in 2002–2008. Although foetal screening has become more popular over time, the prevalence of births in which the neonate has DS has increased in line with the increase in the mean maternal age of the pregnant population. In 2005, the mean maternal age in women giving birth was 30.0 years, and 71 children with DS were born (12.3/10 000 births). In 2008, 81 children with DS were born (13.6/10 000

births) and the mean maternal age was 30.1 years. The mean prevalence of DS at birth was thus approximately 1:813 in 2005, and 1:735 in 2008 (Ritvanen and Sirkiä 2011). In Finland, around 60 000 births occur every year (Vuori and Gissler 2011). With the present screening routine, approximately 75 babies with DS are born each year; it is estimated that without this screening program, there would be approximately 145 DS births each year (Ryynänen et al. 2004). Consequently, the prevalence of DS in newborns between 1993 and 2006 was 1:800, whereas its prevalence without routine screening would be approximately 1:600 (Ritvanen and Sirkiä 2011).

2.3.2 History of Down's syndrome screening in Finland

In the early 20th century it was known that DS was most common in elderly pregnant women, and maternal age was the first metric used in screening for DS. Following the discovery of karyotyping techniques, it was shown in 1959 that DS was caused by triploidy in chromosome 21 (Lejeune et al. 1959). Diagnosis during pregnancy became possible at the end of 1960's, when cells collected from the amniotic fluid could be grown for determination of the chromosomes (Steele and Breg 1966). In Finland, amniotic fluid sampling (AFS) or chorionic villous sampling (CVS) was offered to pregnant women aged over 35–37 years during the 1970's. While the mean age of pregnant women has risen over the last few decades, screening merely based on age does not satisfy the established criteria for screening, since less than half of DS pregnancies occur in women aged 35 years or more (Ritvanen and Sirkiä 2011).

The Finnish National Health Law of 1972 revised the regulations concerning maternal care and follow-up during pregnancy but does not discuss foetal screening (Finnish acts and decrees, 1972). In the 1970´s, ultrasound examinations during the second trimester were introduced to screen for structural abnormalities. Foetal screening based on second trimester serum markers was generalized in a stepwise fashion during the 1980´s. During weeks 15–17, the concentrations of alpha-1-fetoprotein (AFP) secreted by foetal liver and human chorionic gonadotropin (hCG) secreted by placental trophoblasts were measured from maternal serum samples using immunofluorescence (Grenier et al. 1978, Stenman et al. 1983). In the mid 1990's, 93 percent of municipalities offered structural ultrasound examinations and second trimester serum screening was offered in approximately 70 percent of municipalities (Asmala 1995).

AFP and hCG were originally both identified as markers of impaired foetal development (Mishell and Davajan 1966, Seppälä et al. 1973). In a mother with a DS pregnancy, the concentration of AFP is low (Merkatz et al. 1984), while the concentration of hCG is high for the foetus' gestational age (Cuckle et al. 1984, Bogart et al. 1987). Second trimester serum screening detects approximately 60 percent of all pregnancies with trisomy 21 (Haddow et al. 1992), and six to eight percent of screened women are recommended for invasive testing with AFS or CVS. Second trimester screening is not specific for DS, but the advantage of detecting elevated AFP concentrations is that it also makes it possible to identify foetuses with some other major anomalies such as congenital nefrosis (Heinonen et al. 1996a) and spina bifida, i.e. a disturbance in the normal closing of the neural tube (Wald et al. 1977, Norgaard-Pedersen et al. 1985). The disadvantage of second trimester screening is that it is performed at a relatively late stage in the pregnancy; if the results of the screening indicate a high risk of an adverse outcome and invasive testing is needed to verify the chromosome, the pregnancy will be in its 18th or 19th week when the termination is performed. By this point the pregnant woman will be able to sense the foetal movements, and the termination becomes very demanding in both mental and

physical terms. In addition, while second trimester screening works satisfactory in women aged >35 years, its performance is poor for younger women (Spencer 1999, Beaman and Goldie 2001).

Increased nuchal translucency (NT), i.e. a swelling in the back of the foetus' neck, was discovered to be an ultrasound marker for chromosomal disorders in the early 1990's; it becomes apparent between the 10th and 14th weeks of gestation (Nicolaides et al. 1992, Pandya et al. 1994). Traditionally, the practice was that if the NT was more than three millimetres in length, AFS or CVS was offered. It was soon found, however, that not all foetuses with trisomy 21 had such large NTs (Bewley et al. 1995, Hafner et al. 1995). NTs more than three mm are observed in approximately one percent of all pregnancies, and this percent includes 50-70 percent of all instances of trisomy 21, regardless of maternal age (Taipale et al. 1997, Pajkrt et al. 1998). Because of the NT scan's low cost and low screening positive rate, it became the only screening method used by many public health care services in the late 1990's. However, despite the introduction of this test, the prevalence of DS in newborns increased – between 1996 and 1999, DS was observed in 10.0–11.3 / 10 000 newborns, whereas in 2001 it was 14.2 / 10 000 (Ritvanen and Sirkiä 2011). By combining the NT measurement with data on maternal age, and gestational age as determined by the crown-rump length (CRL), 54 to 77 percent of all DS pregnancies could be detected (de Graaf et al. 1999, Bindra et al. 2002, Crossley et al. 2002) with a false positive rate (FPR) of five percent. This demonstrates that optimally carried out measurements can provide results that are about as good as those obtained using second trimester serum screening.

PAPP-A was first identified in the 1970's (Lin et al. 1974), and was subsequently shown to be secreted by placental trophoblasts (Chemnitz et al. 1984). Furthermore, PAPP-A was found to be representative in the first trimester only (Bersinger and Klopper 1984). In early 1990's, a connection between DS and low PAPP-A levels was identified (Cuckle et al. 1992, Brambati et al. 1993). As hCG and fβ-hCG had both been found earlier to be representative in the first and second trimesters (Krantz et al. 1996, Alfthan et al. 1988), first trimester serum screening with fβ-hCG and PAPP-A was estimated to be as sensitive as second trimester triple screening with AFP, hCG and unconjugated estriol, uE3 (Wald et al. 1988, Krantz et al. 1996, Wald et al. 1996a). Estriol is an estrogen hormone and a metabolite of estradiol. It is mainly secreted from the placenta, and is therefore most abundant during pregnancy. In a DS pregnancy, the concentration of estriol is lower than in a normal pregnancy (Canick et al. 1988, Haddow et al. 1990, Cuckle and van Lith 1999).

The detection rate (DR) of the first trimester biochemical markers has been reported to range from 55 to 83 percent with an FPR of five percent (Brambati et al. 1994, Krantz et al. 1996, Cuckle and van Lith 1999, de Graaf et al. 1999, Bindra et al. 2002, Wald et al. 2003, Kagan et al. 2009). Fβ-hCG testing alone detects 28 percent (Wald et al. 2003), and PAPP-A testing alone identifies 44–60 percent of DS pregnancies (Brambati et al. 1993, Wald et al. 2003).

The predecessor of the Finnish National Institute for Health and Welfare (THL), the Centre for Research and Development of Welfare and Health (STAKES), issued a recommendation on foetal screening in 1999 (Viisainen 1999). It was suggested that voluntary screening should be offered to all pregnant women in the public health care system. Structural ultrasound examination was recommended during weeks 16–19 and it was stated that screening for DS should be conducted by means of ultrasound scanning during week 13 or 14 of the pregnancy or by second trimester serum sampling during weeks 15–16. For women aged >39 years, it was suggested that CVS should be offered during weeks 11–12 or AFS during weeks 14–15. In

women aged <35 years, CVS or AFS was recommended for the screening positives only. For women aged 36–38 years, either method could be used. In addition to early ultrasound screening or second trimester serum screening, a second trimester ultrasound could be performed during weeks 18–20 to identify structural abnormalities that would require close monitoring during the pregnancy, such as neural tube defects or renal, urinary, and cardiac anomalies.

According to a survey in 2002 (Terho 2002, Autti-Rämö et al. 2005), the recommendation given in 1999 was not carefully followed in most municipalities. About 90 percent of municipalities offered some sort of screening, but practices varied widely. Responses to a survey of hospitals indicated that the NT scan was the most commonly offered test, being available in 75 percent of all hospitals. First trimester serum screening was available in approximately 15 percent, and the combined FTS was offered by only 10 percent of hospitals.

Serum marker levels and results of the NT scan are independent of one-another in both normal and DS pregnancies. Using both tests in tandem therefore provides a more powerful and accurate risk assessment than can be achieved using either alone. The combined FTS method has a FPR of five percent and its sensitivity ranges from 79 to 92 percent, depending on the fluid tested – whole blood, plasma or serum (Bindra et al. 2002, Malone et al. 2005, Kagan et al. 2008, Kagan et al. 2009) – as well as the maternal age (Orlandi et al. 1997, Krantz et al. 2000, Wapner et al. 2003).

It was shown that the sensitivity and specificity of second trimester screening could be improved by monitoring a fourth serum marker, inhibin A, in addition to the traditional markers AFP and fβ-hCG, and the newer marker uE3 (Aitken et al. 1996, Wald et al. 1996b, Cuckle 2000). Inhibin A is a glycoprotein hormone that develops in the gonads and inhibits sex hormone secretion. It is found in the placenta, and may affect the production of hCG in trophoblasts (Steele et al. 1993). In DS pregnancies, the concentration of Inhibin A is higher than usual (Aitken et al. 1996). Using this quadruple screen, up to 81 percent of pregnancies with trisomy 21 can be detected with a five percent FPR (Gilbert et al. 2001, Wald et al. 2003, Malone et al. 2005). This extended second trimester screen thus performs about as well as the combined FTS.

Ethical factors, practicality, and economic costs should be considered when planning, performing, and developing screens. If DS can be screened during the first trimester, second trimester screening seems obsolete. Between 85 and 90 percent of all foetal aneuploidies can be detected in the first trimester. Following first trimester screening, second trimester serum screening using AFP and fβ-hCG can only identify six percent of the missing 10 to 15 percent of all DS pregnancies. In addition, the number of invasive procedures increases at later stages. It is therefore best to screen as early as possible.

A two-phase integrated screening using both the combined FTS and second trimester quadruple screening would be as sensitive as the combined FTS but would have an FPR of only two percent. A detection rate of 85 percent would be achieved at a FPR of 1.2 percent (Wald et al. 2003); equivalently, up to 96 percent of DS pregnancies would be identified with an FPR of five percent (Wald et al. 2003, Malone et al. 2005). In 2001–2005, large multicenter studies were published comparing different methods of foetal screening. Integrated screening was found to be the most sensitive, most specific and the safest of the tested methods in terms of the risk of miscarriage, but it was also the most expensive and was considered unsuitable for routine use (Gilbert et al. 2001). However, although integrated screening was the most expensive screening method, it was found to be the most cost-effective in terms of determining when invasive tests would be needed (Wald et

al. 2003). Screening based on maternal age and first or second trimester serum screening alone were found to be the least sensitive and specific methods, resulting in the greatest numbers of miscarriages; as such they were the least cost-efficient of the tested techniques (Gilbert et al. 2001). It was concluded that the best methods overall were integrated screening and the combined FTS with NT measurement when performed properly (Gilbert et al. 2001, Malone et al. 2005). In its 2005 report, FinOhta stated that the practical complexity and high cost of the integrated screening method makes it inappropriate for routine use (Autti-Rämö et al. 2005). Table 1 compares the different screening methods in terms of their utility in DS screening and the number of invasive procedures needed to detect one case of DS, according to previous studies.

In 2005, the price of the combined FTS was estimated at circa 150 Euros (Wald et al. 2003, Autti-Rämö et al. 2005). Second trimester screening was judged to cost circa 210 Euros. When structural ultrasound examination was included, the price of the combined FTS was approximately 190 Euros, and the price of second trimester screening was 220 Euros. Using both methods, five percent of screened women will receive a positive risk result, and the price of invasive procedures is the same – circa 650–900 Euros for AFS or CVS (South Savonia Central Hospital District 2011, South Ostrobothnia Central Hospital District 2012). Thus the costs of serum marker screening are independent of the trimester in which the screen is performed, but unsurprisingly, FTS makes it possible to receive the results at a much earlier stage in the pregnancy. Integrated screening was estimated to cost approximately 265 Euros.

To standardise the methods of screening, the Finnish Ministry of Social Welfare and Health updated the Finnish Government Decree on Screenings in 2006. According to the Decree, all pregnant women should be offered a general early pregnancy ultrasound scan during week 10–14, the combined FTS with blood sampling in weeks 8–11 and the NT scan in weeks 10–12, or alternatively, the second trimester triple screening test using AFP, fβ-hCG, and uE3 in weeks 14–15 of the pregnancy. Ultrasound scans should be performed to test for severe structural abnormalities in week 18–21 or after week 24.

To increase the availability of foetal screening in all municipalities, the Finnish Ministry of Social Welfare and Health established a group of specialists in February–December 2008. This group sent out a questionnaire to health care centres in October 2008, which revealed that only 87 percent of municipalities offered the early pregnancy ultrasound scan, even though it should have been in use in each centre by January 2007. The combined FTS was offered by 79 percent. In addition, structural ultrasound during weeks 18–21 was offered by 83 percent, and after week 24, only by 35 percent (The Ministry of Social Welfare and Health, 2009).

The Finnish Government Decree on Screenings during pregnancy was revised in 2009. Under the new Decree, every municipality had to offer voluntary screening to pregnant women by the year 2010. All pregnant women must be offered the first trimester general ultrasound scan in weeks 10+0 – 13+6; chromosomal disorders must be primarily screened using the combined FTS with blood sampling at weeks 9+0 – 11+6 and the NT measurement at weeks 11+0 – 13+6, or using the second trimester screening with blood sampling at weeks 15+0 – 16+6; and structural ultrasound examination must be offered at weeks 18+0 – 21+6 or after week 24+0.

Table 1. The performance of different DS screening methods and the number of invasive procedures subsequently required, as reported in previous publications

Study	N	Mean age (yrs)	Incidence of DS (%)	FPR (%)	DR (%)	NIPN	FPR (%)	DR (%)	NIPN	FPR (%)	DR (%)	NIPN
First trimester screening				fβ-hCG and PAPP-A			NT screening			Combined screening		
Krantz et al. 1996	505	28.2		5.0	68.2							
Orlandi et al. 1997	2 010	32	0.55	5.0	61	10	5.0	73	6	5.0	87	10
Taipale et al. 1997*, **	10 010	29	0.13				0.8	54	11			
Pajkrt et al. 1998**	1 473	31.4	0.61				2.2	67	3.6			
de Graaf et al. 1999	300			5.0	55		5.0	68		5.0	85	
Krantz et al. 2000	10 251	31.8	0.49							5.0	91	11
Gilbert et al. 2001	95 802			5.0	63		5.0	74		5.0	86	
Niemimaa et al. 2001	2 515		0.83	5.0	75	21	5.0	60	26	5.0	80	20
Schuchter et al. 2001	5 012	30	0.28				4.8	57	29	5.0	86	20
Bindra et al. 2002	14 383	34	0.57	5.0	60	15	5.0	79	10	5.0	90	10
Crossley et al. 2002	17 229	29.9	0.26	5.0	55	37	5.0	54	31	5.0	82	30
Spencer et al. 2003	12 339	30	0.20							5.2	92	35
Wald et al. 2003	47 053	29	0.21	5.0	83	24	5.0	60	24	5.0	93	23
Wapner et al. 2003	8 514	34	0.72	5.0	67	10	5.0	68.8	10	5.0	79	9
Malone et al. 2005	36 120	27.1	0.25							5.0	87	23
Nicolaides et al. 2005	75 821	31	0.43							5.0	90	13
Kagan et al. 2008	56 376	35.4	0.70							5.0	94	8
Kagan et al. 2009	19 736	34.5	0.62	5.0	67	12	5.0	82	10	5.0	93	9
Second trimester screening				AFP and fβ-hCG			Triple screening			Quadruple screening		
Wald et al. 1988	462			5.0	55					4.7	60	
Haddow et al. 1992	25 207	27.0	0.14				5.3	58	38			
Spencer 1999	67 904	29	0.16	5.1	75	43						
Beaman and Goldie 2001	66 631	28.2	0.16	5.8	66.7	38						
Gilbert et al. 2001	95 802			5.0	60		5.0	68		5.0	79	
Wald et al. 2003	47 053	29	0.21	5.0	66		5.0	88		5.0	90	25
Malone et al. 2005	35 236	27.1	0.31							5.0	81	25
Integrated screening				PAPP-A and NT + quadruple screening			PAPP-A + triple screening			PAPP-A + quadruple screening		
Gilbert et al. 2001	95 802			5.0	95							
Wald et al. 2003	47 053	29	0.21				5.0	92	19	5.0	93	20
Malone et al. 2005	33 546	27.1	0.25	5.0	96	20				5.0	88	22

NIPN = number of invasive procedures needed to detect one case of DS
Triple screening = AFP, fβ-hCG, and uE3
Quadruple screening = AFP, fβ-hCG, uE3, and inh A
*gestational age range 10+0 – 15+7 with the median 13+4, ** screening positive: NT ≥ 3 mm

2.4 THE FIRST TRIMESTER COMBINED SCREENING METHOD

In a DS pregnancy, the foetus and especially the placenta mature and develop more slowly than they would in a healthy pregnancy. Screening aims to detect this protracted placental development, whose primary markers are unusually high levels of fβ-hCG and NT, and low levels of PAPP-A.

The FTS method combines the maternal serum markers – fβ-hCG and PAPP-A – with NT measurement of the foetus. The maternal serum samples are collected at maternity care units during gestational weeks 9+0 – 11+6. The samples are frozen and delivered to the analysing laboratory, where the concentrations of fβ-hCG and PAPP-A are measured using a time-resolved fluoroimmunoassay. NT and CRL are measured at health care centers and maternity clinics by ultrasound-trained midwives and gynaecologists between weeks 11+0 and 13+6.

Screening in Finland is centralized in major accredited laboratories using the same method. Commonly 40–50 municipalities pay for FTS to be performed at a laboratory center or the laboratory of the central university hospital district. A pregnancy-specific DS risk ratio is calculated in the analysing laboratory, based on the concentrations of fβ-hCG, and PAPP-A, and the NT measurement in millimetres. The calculated pregnancy-specific risk ratio either increases or decreases the maternal age risk ratio for DS pregnancy. A cut-off value of 1:250 is used – the risk is considered normal when the probability of having a foetus with DS is estimated at less than 1 in 250, whereas the risk is considered higher than normal if the probability is estimated greater than 1 in 250.

2.4.1 Risk calculation in the first trimester screening

Risk calculation is performed in the laboratory, where all of the information concerning the mother is gathered and entered to the risk calculation software LifeCycle (PerkinElmer LifeSciences, Wallac, Turku, Finland). The name and social security number of the pregnant woman, the date on which the blood sample was collected, the mother's weight, their history of smoking and previous DS pregnancies, the date of the beginning of their last menstrual period, and any medication they are taking, are received from the maternity care unit or the maternity clinic. The concentrations of fβ-hCG and PAPP-A are obtained using the Auto-DELFIA fluoroimmunoassay system. The NT and CRL measurements, the number of foetuses, and the date of the ultrasound scan are received from the maternity clinic.

In the risk calculation software, the patient's results of fβ-hCG, PAPP-A, and NT are compared to a population model described by a set of multivariate Gaussian distributions. The risk calculation programme provides a multiple of a median (MOM) value for each marker, proportional to gestational age. A MOM value is a measure of how far an individual test result deviates from the median of the population. The three markers, fβ-hCG, PAPP-A, and NT, are mathematically transformed into MOM values, by comparing the given value in this specific pregnancy with the median value for a normal pregnant population. For each median, a minimum of 50 values at each gestational week has been stored for comparison in the software. The MOM value is the measured value for the patient divided by the median for the statistic population. In risk calculation, medians are used rather than means because using means would distort the statistics, causing the distribution of values to lean to the left; in other words, the data would include more the small than the large values.

In the overall population, the individual corrected MOM values for normal pregnancies should be 1.0. The median MOMs in DS pregnancies have been reported as 1.16–2.48 for fβ-hCG, 0.34–0.63 for PAPP-A, and 1.43–2.34 for NT (Cuckle and van Lith 1999, de Graaf et al. 1999, Crossley et al. 2002, Wald et al. 2003, Malone et al. 2005, Koster et al. 2011), depending on the gestational age. These medians may need correction, as they vary by ethnic population, maternal and gestational age, maternal weight (Wapner et al. 2003, Malone et al. 2005, Kagan et al. 2009), the smoking habits of the pregnant woman (Lambert-Messerlian et al. 2009), whether the pregnant woman suffers from insulin-treated diabetes (Wald et al. 1994, Spencer et al. 2005), and the origin of the pregnancy – artificial or spontaneous (Gjerris et al. 2009, Kagan et al. 2009). Based on modern knowledge, no adjustments for maternal age or origin of the pregnancy are required. Version 2.1 of the LifeCycle risk calculation software package was used in the Eastern Finland Laboratory Centre and Kuopio University Hospital prior to May 2008. In this version, which was also used to analyse the data examined in this work, the concentrations of both fβ-hCG and PAPP-A were corrected for maternal weight, gestational age, and diabetes, but only the fβ-hCG concentration was corrected for smoking.

The risk calculation programme provides the likelihood ratio for DS based on maternal age, as well as the pregnancy-specific risk ratio based on the median MOMs of the combined screening markers and maternal age. The likelihood ratio provides an estimated probability that the foetus will have DS relative to the estimated probability of having a foetus with a normal karyotype. The possible risk ratio range with LifeCycle 2.1 is 1:5–1:50 000. A cut-off value of 1:250 is used to distinguish between screening positives (for which the estimated risk ratio is >1:250) and screening negatives (estimated risk ratio <1:250). This cut-off gives a fixed FPR of approximately five percent, i.e. there is a five percent chance that a screened woman will have a positive risk estimate. The likelihood ratio of 1:250 is comparable to the risk that an average 35 year old woman will have a baby with DS. The actual screening positive rate (or equivalently, the FPR) for the Eastern Finland Laboratory Centre between 2002 and 2008 was 3.7 percent since the median age of the screened population was 29.3 years.

If the pregnancy-specific risk ratio is considered normal (i.e. <1:250), the analysing laboratory will report the likelihood ratio to the maternity clinic or maternity care unit by mail, and the pregnant woman will be informed at her next visit. However, if the likelihood ratio is higher than 1:250, the result will be sent both by fax and by mail to the maternity clinic, which will direct the pregnant woman to medical genetics counselling and possible invasive testing with AFS or CVS.

In each series of sample measurements, a calibration curve is ran for internal quality control. The calibration reports are frequently examined by the hospital chemist in charge. For external quality control, supervised by an international quality assurance company (UK NEQAS, Edinburgh, Great Britain), the concentrations from quality control samples are measured and their MOM values as well as the specific DS risk ratio are calculated and sent to the quality control company, produce a report on the quality of the results.

2.4.2 Free beta subunit of human chorionic gonadotrophin

In addition to being a marker for pregnancy, hCG is used as a marker for chorionic (Smith et al. 2005) and testicular cancers (Klein 1993). HCG is a glycoprotein consisting of two subunits α (fα-hCG) and β (fβ-hCG), which can either be non-covalently bound to each other or exist as free subunits in the serum. HCG secreted by the placental trophoblasts appears in the circulation approximately one week after

conception and reaches its peak levels at approximately 8–12 gestational weeks. High levels of fβ-hCG are particularly common in DS pregnancies (Spencer et al. 1992). This protein is therefore most useful as a marker for DS screening in weeks 9-12 (Cuckle and van Lith 1999, Wright et al. 2010).

2.4.3 Pregnancy associated plasma protein A

PAPP-A is a placental glycoprotein produced by the trophoblasts. Small amounts of PAPP-A are also present in non-pregnancy associated tissues such as the liver, heart, and kidney. High PAPP-A concentrations have been linked with asthma (Coskun et al. 2007), coronary artery disease, and an increased risk of myocardial infarction (Iversen et al. 2011), but the protein's biological functions remain largely unknown. PAPP-A is known to be an insulin-like growth factor binding protein (IGFBP) protease and to increase the bioavailability of insulin-like growth factor (IGF). IGF, in turn, is believed to play a role in foetal growth (Lawrence et al. 1999, Bale and Conover 2005) by mediating trophoblast invasion into the decidua, and by regulating steroidogenesis and glucose and amino acid transport in the chorionic villi (Sun et al. 2002). PAPP-A participates in growth regulation, and is therefore probably related to foetal development. In DS screening, PAPP-A is representable only during the early stages of pregnancy in weeks 0–13, because its serum concentration increases physiologically in later weeks as the pregnancy proceeds. PAPP-A is most useful for DS screening in week 9 (Cuckle and van Lith 1999, Tørring 2009).

2.4.4 Pre-analytical phase

The maternal venous blood samples are collected in health care centre laboratories. Blood (approximately three mL per sample) is collected by venipuncture into serum tubes, allowed to clot at room temperature for 30–90 minutes, and centrifuged at 2000–3000 G, allowing 10 minutes for separation. The sera are stored in a refrigerator at four degrees of Celsius and are delivered to the analytical laboratory cold or frozen; they are stored at –20 degrees of Celsius. If a room temperature sample is received by the laboratory, it is processed normally, but the delivery temperature of the sample is noted when writing the report, and the validity of the result is estimated by a physician.

2.4.5 Analytical phase

In the Eastern Finland Laboratory Centre, the concentrations of fβ-hCG and PAPP-A are measured using a Wallac AutoDELFIA immunoanalyser (PerkinElmer, Turku, Finland) and ready-made reagents. The reagents are kept at a temperature of + 2 – - 4 degrees of Celsius. Before the analysis, the standards are prepared and wash solutions for the plate and sample processors are poured. The samples are left to stand and thaw at room temperature for one and half hours prior to analysis. A full standard curve is run for each assayed plate. The information on samples and controls is entered on the work list in the AutoDELFIA workstation software. The samples, controls, and standard vials are then loaded and a schedule for the series is drawn up. The bar codes on the test tubes are read by the analyser, which measures the concentrations of each marker, and prints out the results (PerkinElmer 2008).

During the analysis, fβ-hCG is attached to a samarium-labelled monoclonal antibody, i.e. an anti-fβ-hCG antibody produced by a single stem cell clone, whereas PAPP-A is attached to a europium-labelled monoclonal anti-PAPP-A antibody. The greater the concentration of fβ-hCG and PAPP-A in the sample, the greater the quantity of the fluorescent label that is bound. The concentrations of each marker are directly proportion to the intensity of the fluorescence emitted by the labelled

antibodies. The fluorescent label begins fluorescing after being displaced from the labelled antibody by the measuring solution and the label forms a chelate (PerkinElmer 2008).

Fβ-hCG concentrations are reported in ug/L; the measured and reported concentrations range from 2–200 ug/L. PAPP-A concentrations are reported in mU/I (arbitrary units); PAPP-A measurements range from 10–2000 mU/I, and the results range from 50–10 000 mU/I. The lowest limits of detection for fβ-hCG and PAPP-A are 0.2 ng/mL and 5 mUI/L, respectively (PerkinElmer 2008). If the measured concentration in the sample exceeds the highest value standard, the sample must be diluted using the DELFIA Diluent II solution; typically, a 1:10 dilution is prepared in the first instance. The sample is then re-analyzed and the result is multiplied by the dilution coefficient to determine the true concentration.

AutoDELFIA and DelfiaXpress systems, which yields similar results to those obtained with AutoDELFIA, are used in Finnish laboratories, but other assay systems are also available, including Kryptor (Brahms AG, Henningsdorf, Germany) and Immulite (Siemens Healthcare Diagnostics., Deerfield, IL, USA) immunoassay systems, which give measured PAPP-A concentrations that are typically twice as high as those measured with AutoDELFIA. This, naturally, is accounted for in the risk calculation algorithm.

2.4.6 Post-analytical phase

The AutoDELFIA time-resolved fluoroimmunoassay kit exhibits both within- and between-assay variation. Both are below 3.4 percent for fβ-hCG at concentrations of 4–157 ug/L, while the corresponding values for PAPP-A are <2.4 and <4.0 percent, respectively, in the detection range of 44–7300 mUI/L. Internal quality control is performed by calibration before each assay. External quality control is supervised by an international quality assurance company (UK NEQAS, Edinburgh, Great Britain). The quantification of fβ-hCG and PAPP-A is very accurate and reproducible. The most important sources of error in the biochemical screening relate to sample collection and storage (Palomäki et al. 2009, Lambert-Messerlian et al. 2006).

2.4.7 Ultrasonic nuchal translucency measurement

Foetal NT, otherwise known as foetal neck oedema – the unusually thick layer of fluid imaging with a low grade echo – is associated with chromosomal abnormalities, cardiovascular defects (Hyett et al. 1996), skeletal dysplasias (Hyett et al. 1997, Khalil et al. 2011), infectious diseases during pregnancy (Sebire et al. 1997), and some rare genetic syndromes. The accumulation of foetal subcutaneous fluid is referred to as nuchal translucency in the first trimester, whereas in the second trimester it is called a nuchal fold. It should ideally be measured in weeks 11+0 – 13+6 because NT is typically seen during weeks 10–14 but disappears afterwards. NT is routinely measured by ultrasound during pregnancy weeks 11–13, with the measurement being made transabdominally or transvaginally; the results obtained using both methods are congruent and the measurement is successful in 95 percent of women (Whitlow et al. 1999, Nicolaides, 2004).

NT is measured in the back of the foetal cervical spine, from the external border of soft tissue to the internal border of the skin (Figure 1). The greatest possible magnification should be used during the measurement, and the image is magnified so that the foetus fills approximately two thirds of the monitor. Scanning is maintained during spontaneous foetal movement in order to attain the best possible sagittal scan position and thus the 'view' that best distinguishes between the skin and the foetal membranes. The umbilical cord may be wrapped around the neck,

causing its apparent size to increase by up to 0.8 millimetres; when wrapped around in this way, the umbilical cord can be misinterpreted as NT. NT is dependent on the gestational age, which is determined by measuring foetal CRL. Using CRL, gestational age can be determined with an accuracy of ± three days (Taipale and Hiilesmaa 2001). CRL measurement is recommended for foetuses whose length is less than 60 millimetres. If the foetus is taller than this, the biparietal diameter or femur length should be used.

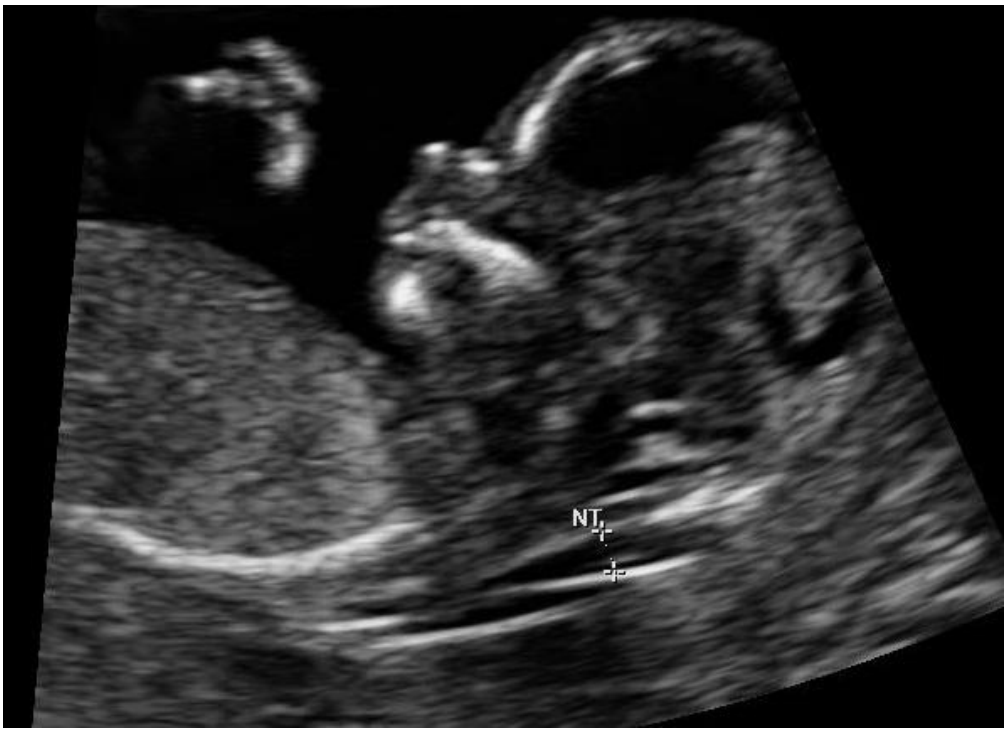

Figure 1. NT measurement of a foetus during the first trimester. The nasal bone can also be seen (Department of Gynaecology and Obstetrics, Kuopio University Hospital).

NT is the most efficient ultrasonic measure for aneuploidies. The MOM of NT is not dependent on maternal weight (Gilbert et al. 2001) or maternal age (Taipale et al. 1997), unlike the MOMs of the biochemical markers. However, a foetus with DS might seem completely normal in **a** structural ultrasonic examination, and only 50–70 percent of affected foetuses exhibit extensive NT (Taipale et al. 1997, Pajkrt et al. 1998). NT increases with the duration of the pregnancy; the mean NT in normal pregnancies at week 9 is approximately 0.7 mm, rising to 1.2 mm in week 10 and 1.5 mm in week 13. According to our records, in DS pregnancies the mean NT at week 10 is 1.8 mm, rising to 2.4 mm in week 11, 2.9 mm in week 12, and 3.2 mm in week 13. Consequently, the average MOM value for DS pregnancies is approximately 2: 1.9–2.0 at week 10, 1.8–2.1 at week 11, 1.6–2.5 at week 12, and 1.4–2.0 at week 13 (Crossley et al. 2002, Wald et al. 2003, Malone et al. 2005, Koster et al. 2011).

High NT values are sometimes observed in chromosomally normal foetuses. In cases where a large NT is observed by but the chromosomes are shown to be normal by AFS or CVS, the foetus has an increased risk of congenital heart defects and many

other anomalies (Souka et al. 1998, Nicolaides et al. 2002, Mendoza-Caamal et al. 2010). The most probable aetiology of NT in both euploid and aneuploid foetuses is heart failure due to abnormal or delayed embryonic and foetal development of the heart and other organs. The observation of a large NT therefore requires that a structural and cardiac ultrasonic scan be performed by a specialist.

The ultrasonic measurement of NT has been shown to be the most challenging component of the combined FTS. In Finland, ultrasound-trained midwives usually perform the measurements, whereas in many other countries the first trimester ultrasound scans are often carried out by gynaecologists or radiologists. The time reserved for each pregnant woman per scan in Finland is usually 20 minutes, whereas 30 minutes is common abroad. However, it has been estimated that it takes three years and approximately 700 measurements for a trained midwife to achieve adequate precision and repeatability (Taipale et al. 2003). To ensure the quality of the measurements, it is recommended that each practitioner conduct at least 200 each year (Tekay 2009).

2.5 SPECIAL SITUATIONS IN FIRST TRIMESTER SCREENING

2.5.1 Multiple pregnancies

Screening of twin pregnancies is problematic. In normal twin pregnancies, the concentrations of the serum markers are approximately twice as high as in normal singleton pregnancies. Unusually high or low marker concentrations in a screened twin pregnancy indicate a risk of adverse events, but it is impossible to say which of the foetuses may have a chromosomal abnormality, especially if their NTs are normal (Spencer and Nicolaides 2003, Audibert and Gagnon 2011).

The ultrasonic measurement establishes whether the pregnancy is singleton or multiple. In twin pregnancies, observations are conducted to determine whether the pregnancy is mono- or dichorial, in other words whether the foetuses have a common foetal chorionic membrane or whether each has one of their own. Determination of chorionity is extremely meaningful because monochorial pregnancies are inescapably monozygotic, i.e. the foetuses are identical twins and originate from the same oozygote, whereas in dichorial pregnancies, 90 percent of the foetuses are dizygotic and originate from two different eggs. In dizygotic pregnancies, the risk of trisomy is doubled due to twinning, whereas in monozygotic pregnancies the age risk is the same as in singleton pregnancies, but either with both foetuses having a normal or an abnormal karyotype. NT measurement remains useful as a screening method in twin pregnancies whereas serum markers are merely directional (Spencer 2000, Spencer and Nicolaides 2003). On the other hand, if chorionity is assured by ultrasound, biochemical markers improve the DR as compared to NT measurement alone, and can be used advisedly in multiple pregnancies (Audibert et al. 2011, Prats et al. 2012).

2.5.2 Assisted reproductive technology

It has been reported that the levels of all three markers used in the combined FTS are changed in assisted reproductive technology (ART) pregnancies, but there is no evidence that the frequency of DS is higher in pregnancies induced using assisted reproduction than in those with spontaneous origin (Liao et al. 2001, Hui et al. 2006, Gjerris et al. 2009). The median fβ-hCG MOM level in chromosomally normal IVF pregnancies has been shown to range from 0.84–1.21 depending on the gestational age, while the median PAPP-A MOM in these pregnancies has been reported to range from 0.75–1.0 (Orlandi et al. 2002, Ghisoni et al. 2003, Tul and Novak-Antolic

2006, Anckaert et al. 2008, Amor et al. 2009, Gjerris et al. 2009, Kagan et al. 2009). The median NT MOM values have ranged between 1.0–1.6 MOM (Liao et al. 2001, Orlandi et al. 2002, Hui et al. 2006, Gjerris et al. 2009). Hence, the estimates of these markers tend to increase the FPR in ART pregnancies (Heinonen et al. 1996b, Orlandi et al. 2002, Tul and Novak-Antolic 2006, Amor et al. 2009, Gjerris et al. 2009, Kagan et al. 2009).

2.5.3 Other aneuploidies than Down's syndrome

Besides DS, the combined FTS can be used for screening of other major trisomies such as trisomies 13 and 18 (Spencer and Nicolaides 2002, Wapner et al. 2003, Breathnach et al. 2007, Kagan et al. 2008). During the first trimester, the MOM of fβ-hCG in trisomies 13 and 18 has been reported as 0.16–0.41, the MOM of PAPP-A as 0.0.9–0.36, and the MOM of NT 1.17–2.82 (Spencer and Nicolaides 2002, Breathnach et al. 2007, Kagan et al. 2008). Since version 2.1, the LifeCycle risk calculation software has had a standard algorithm for calculating the risk ratio for trisomy 18.

2.5.4 Anomalies

Foetal structures are inspected in early sonographic examinations and during the NT measurement. Anomalies such as an absent umbilical cord, a large ventral wall defect with herniation (Murphy and Platt 2011), or the absence of a limb can be detected during these inspections (Rice et al. 2011). There is no clear evidence indicating that such anomalies are associated with any alteration of first trimester biochemistry (Aitken et al. 1993), although there is a reported link between an increased risk ratio in the combined FTS and the risk of anomalies, such as abnormal ductus venous flow and tricuspid regurgitation (Ozkaya et al. 2009).

2.5.5 Miscarriages

If during the first trimester approximately five percent of pregnancies are miscarried, three percent of women who undergo the combined FTS would have blood samples taken but would then have their pregnancies spontaneously aborted before the NT scan, and two percent of those who have all three markers of the combined FTS measured, would undergo spontaneous abortion after the combined FTS. Furthermore, some pregnancies are spontaneously aborted after CVS or AFS – some of these are due to the invasive testing and some would have been aborted anyway. In cases of miscarriage, the laboratory will not receive the diagnosis of the aborted foetus, and so the true DR from screening is unknown.

2.5.6 Adverse pregnancy outcomes

It has been observed that the concentrations of fβ-hCG and especially PAPP-A are lower than normal for adverse pregnancy outcomes with a normal foetal karyotype, such as PD, PE, and SGA (Smith et al. 2002, Tul et al. 2003, Dugoff et al. 2004, Liu et al. 2004, Brameld et al. 2008, Spencer et al. 2008a, Spencer et al. 2008b, Poon et al. 2009). Low levels of fβ-hCG and PAPP-A probably reflect impaired placentation. There has been some discussion on whether such pregnancies should be monitored closely. To date, no risk calculation algorithms for adverse pregnancy outcomes have been established.

3 Diagnosis of chromosome disorders

3.1 INDICATIONS FOR CHROMOSOME DETERMINATION

When screening indicates that a pregnant woman is subject to an increased risk of DS, she and her family are given an appointment for medical genetics counselling. If the expectant family is willing, the foetal chromosomes are tested by AFS or CVS. The sampling takes place at the maternity clinic. Chromosome determination is performed in the department of clinical genetics, which is also in charge of genetic counselling if the foetal chromosome is found to be abnormal.

Aside from a high risk ratio in foetal screening, other indications for chromosome analysis are high maternal age – age limits are municipality-specific and range from 35 to 40 (Viisainen 1999), a known chromosomal abnormality in either parent or one of their older children, and the detection of a structural development disorder by ultrasound. Such structural abnormalities include ventral hernia, talipes equinovarus (i.e. clubfoot deformity), heart defects, and other foetal abnormities such as an NT above three millimetres, plexus chorioideus cyst, dilated renal pelvises, emphasized intestinal echo or increasing IUGR.

3.2 METHODS FOR CHROMOSOME DETERMINATION

The choice of whether to use AFS or CVS depends on gestational age. Invasive procedures to determine the foetal chromosome necessitate a transabdominal puncture with a thin needle to the uterus. In AFS, there is a 0.5 percent risk of miscarriage, and in CVS, the risk is estimated to be 0.5-1.0 percent (Tabor and Alfiveric 2010). The aspired sample is injected onto a Petri dish and taken to the laboratory of clinical genetics. The sample is then processed and labelled for immunofluorescence. The chromosomes are identified and counted under a fluorescence microscope, and a chromosomal map is made for examination (Figures 2a and 2b). Certain chromosomes can be detected by fluorescent *in situ* hybridization using gene probes. Chromosome determination by fluorescent *in situ* hybridization is very reliable: over 99 percent of examinations lead to an accurate result. Chromosomal mosaicism can cause uncertainty because it means that the sample will contain both chromosomally normal and chromosomally abnormal cells. If the CVS data indicates mosaicism, an AFS sample is taken. AFS is more reliable and generates mosaic results less frequently than CVS. If AFS sampling indicates mosaicism, an appointment with a specialist in medical genetics is arranged to discuss the finding.

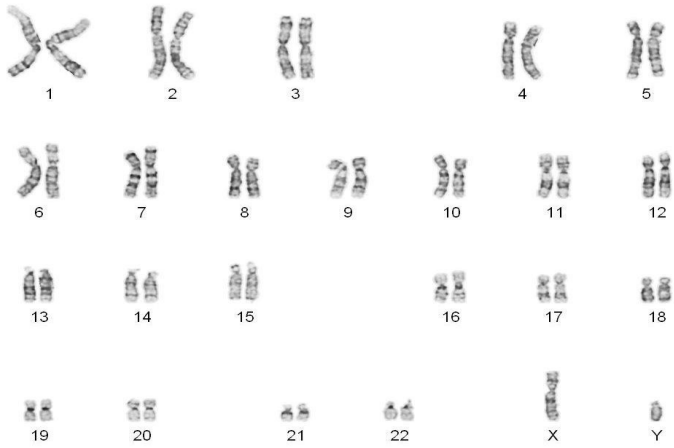

Figure 2a. A normal euploid chromosome of a male foetus (46,XY) (Laboratory of Clinical Genetics, Eastern Finland Laboratory Centre, Kuopio, Finland).

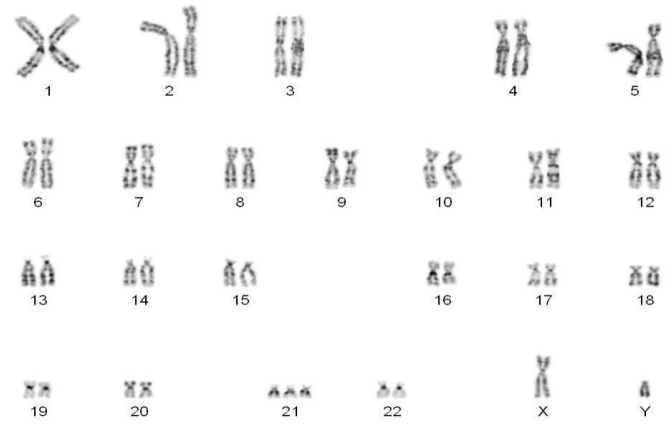

Figure 2b. A chromosome of a male foetus with Down's syndrome (47,XY,+21) (Laboratory of Clinical Genetics, Eastern Finland Laboratory Centre, Kuopio, Finland). Both chromosomes in Figures 2a and 2b are taken from an amniotic fluid sample during early second trimester.

CVS can be performed after the 10th gestational week. The placenta is punctured using ultrasonic guidance, and placental tissue is aspired. The sample is incubated in a thermic cabinet at 37 degrees of Celsius, and frequently examined under a microscope to observe cell division. Mitosis is usually observed in less than a week, and the chromosomes are stained so that they can be identified and counted under a microscope. If no dividing cells are clearly observed, the sample is cultivated and the result is given in 3–4 weeks. In practice, CVS is usually taken after the 11th week, and a result is obtained in 5–7 days.

AFS is usually performed in week 15 or 16 of gestation. The sample is aspired from the uterine cavity, where the needle can be seen by ultrasound. The amniotic fluid always includes some foetal cells, and the cells are cultivated for chromosome determination. Completion of the result typically takes two or three weeks. The concentration of AFP is routinely measured from the amniotic fluid to monitor foetal structural abnormalities that might not have been seen in ultrasonic measurements, such as spina bifida or congenital nefrosis.

Cordocentesis i.e. foetal blood sampling from the umbilical chord is not a recommended option for determination of the foetal chromosome, since the risk of miscarriage is as high as 15 percent (Bernaschek et al. 1995). However, if there are

justifiable reasons for sampling in this way, the sample can be collected at any point before week 18 of gestation.

3.3 FURTHER ACTIONS AFTER DIAGNOSIS

If AFS or CVS indicate that the foetus has chromosomal abnormalities, the parents should be offered medical genetics counselling or a meeting with a perinatologist. Counselling is given to ensure they understand the meaning of the particular abnormal karyotype, and to allow them to plan their actions. If the parents decide to continue the pregnancy, follow-up monitoring during the pregnancy, delivery, and possible care of the newborn should be organized as best possible. If the parents decide to terminate the pregnancy, they can apply for termination from the National Institute of Health and Welfare. Permission for termination can be granted on the grounds of foetal developmental disorder or disability at any point up to the 24th week of gestation. If the pregnancy is terminated due to a foetal developmental disorder, the nature of the disorder is verified by examining and photographing the aborted foetus, examining its chromosomes, and performing an autopsy. Genetic counselling can thus provide the parents with information on the likelihood that the abnormality will affect any future pregnancies they may have and ensure they receive proper care during any later pregnancy.

4 Aims of the study

The studies reported in this thesis were conducted to investigate the factors influencing the concentrations of the biochemical markers in the combined FTS.

A key objective was to examine the effects of the TTP on the first trimester DS markers in spontaneous, chromosomally normal pregnancies, and to compare the results to those in IVF pregnancies. Our main interest was in the concentrations of fβ-hCG and PAPP-A in pregnancies with a prolonged TTP.

Previous studies have reported altered concentrations of fβ-hCG and PAPP-A in pregnancies with PD, PE, and SGA. We investigated the profile of the biochemical markers in adverse pregnancy outcomes, including PA, in chromosomally normal pregnancies.

The concentrations of fβ-hCG and PAPP-A vary with maternal age, and the DR of the combined FTS has been known to be higher in women aged >35 years than in women aged ≤35 years. The numbers of invasive procedures needed to detect one DS pregnancy in women between 15 and 48 years of age were examined according to 5-year age groupings.

Finally, the performance of the first trimester biochemical screening markers at different maternal ages was analysed.

5 Material and methods

5.1 STUDY DATA

The investigations were conducted using case-control studies. Screening data were collected in the Eastern Finland Laboratory Centre, Helsinki Central University Hospital Laboratory Centre, Oulu University Hospital Laboratory, and Seinäjoki Central Hospital Laboratory. The maternal and pregnancy data were collected from the Departments of Gynaecology and Obstetrics of Helsinki, Kuopio, and Oulu University Hospitals, as well as Seinäjoki Central Hospital. The results of chromosome examinations were collected from each laboratory centre's or hospital's Department of Clinical Genetics. The data used in the study are presented in Table 2.

Table 2. Data used in the study

Work	Data source	N	Follow-up	Study design	Pregnancy karyotype	Study problem
I	Kuopio University Hospital	1 385	Jan 2005 – Dec 2007	Observational case-control	Normal	Time to pregnancy vs. fβ-hCG and PAPP-A concentrations
II	Kuopio University Hospital	2 844	Jan 2005 – Dec 2007	Retrospective case-control	Normal	Adverse pregnancy outcome vs. fβ-hCG and PAPP-A concentrations
III	Helsinki, Oulu, and Kuopio University Hospitals; Central Finland, Kymenlaakso, Mikkeli, North Carelia, and Seinäjoki Central Hospitals	65 600	May 2002 – Dec 2008	Retrospective case-control	DS or normal	Maternal age vs. the performance of the combined screening
IV	Helsinki, Oulu, and Kuopio University Hospitals; Central Finland, Kymenlaakso, Mikkeli, North Carelia, and Seinäjoki Central Hospitals	76 949	May 2002 – Dec 2008	Retrospective case-control	DS or normal	Maternal age vs. the performance of the biochemical screening

In studies I–II, the investigated factors hypothesized to influence on the combined FTS were: maternal age, origin of the pregnancy (spontaneous/IVF), the TTP in

spontaneous pregnancies, and the outcome of the pregnancy (normal/PA/PD/PE/SGA). In work I, spontaneous pregnancies were classified into three groups by TTP: 0–12 months (the reference group, N=1164), 13–24 months (N=112) and ≥25 months (N=70). Screening data from IVF pregnancies were collected for comparison (N=39). The size of the total study population was 1385. In work II, the four study groups were pregnancies with PA (N=17), PD (N=213), PE (N=175), and SGA (N=275) plus a reference group with a normal outcome (N=2164). Reference pregnancies were taken to have a normal outcome if the foetus had an euploid chromosome, the pregnancy duration was at least 38 weeks, and the mother and newborn did not require any prenatal, perinatal or postnatal follow-up, care or intervention over and above that administered by routine. The size of the total study population in this work was 2844. The sample size in study I was smaller than that in study II since the number of IVF pregnancies with no missing data was only 39, and TTP is not typically requested in patient-reported information.

In studies III–IV, pregnancies with euploid karyotypes were used as references, and singleton pregnancies with DS were considered cases. In the combined FTS, the number of DS pregnancies was 188; and in biochemical screening the number of DS pregnancies was 221, so there were 33 cases without NT data. The size of the entire screened population was 76 949, of which 65 600 had been screened using all three markers, including NT. The results were compared in 5-year age groups (≤19 years, 20–24 years, 25–29 years, 30–34 years, 35–39 years, 40–44 years, and ≥45 years).

5.2 PATIENT DATA

The data for studies I–II was collected from the same pregnant population in the Kuopio catchment area. Screening and follow-up data on the index pregnancy were collected between January 1 2005 and December 31 2007 from the databases of the Eastern Finland Laboratory Centre, and the Department of Gynaecology and Obstetrics in Kuopio University Hospital. The results from the multicentre study were published in papers III–IV. The information was collected in Helsinki, Kuopio, Oulu, and Seinäjoki between May 1 2002 and December 31 2008.

The patient-reported information included the weight and height of the mother, information on whether she had any chronic diseases (hypertension or diabetes), her smoking habits (yes/no), the waiting time to pregnancy measured from the start of attempts to become pregnant to the observation of an actual pregnancy, possible prior pregnancy complications, and whether she had undergone infertility treatment. Systematically gathered information included the FTS results (the age of the mother, maternal serum fβ-hCG and PAPP-A concentrations and the NT of the foetus), the method of conception (spontaneous or IVF), –, body mass index (BMI) before and at the end of the index pregnancy, pregnancy duration, possible pregnancy complications, pregnancy outcome (normal/abnormal chromosomes, and the karyotype; PA/PD/PE/SGA), the method of chromosome determination (AFS/CVS), gender of the foetus, and birth weight. PE was indicated in cases where blood pressure measurements above >140/90 mmHg were recorded on multiple occasions, with proteinuria >0.3 g/day. PD was indicated by births occurring before the end of the 37th week of gestation. SGA was indicated by the sex- and age-adjusted birth weight falling within the 10th percentile according to our records, and PA was diagnosed clinically.

5.3 THE SCREENING METHOD

Screening was conducted using the combined FTS method. Serum samples were collected between weeks 8+0 and 12+6, and NT measurements were conducted between weeks 10+0 and 13+6. The maternal biochemical data were analyzed retrospectively. For studies I-III, this meant using the original DS risk ratios from the combined FTS. However, in study IV, data from the population examined in study III were analyzed, focusing exclusively on information on the serum markers fβ-hCG and PAPP-A; the DS risk ratios for the biochemical screening were re-calculated using the combined screening data with NT results excluded from the risk assessment.

5.4 ETHICS

The study was approved by the Ethical Research Committees of Helsinki, Kuopio, and Oulu University Hospitals, and the Institutional Review Boards. The study was planned to examine the quality of the combined FTS, and thus provide information for internal quality control. The Committees gave permission for the results to be published. In addition, permission to access the national registers of births and newborns, induced abortions, and anomalies, was sought from the Ministry of Welfare and Health and STAKES in Autumn 2006, and granted a year later for 2006–2009. The combined FTS was performed according to the recommendations of the Finnish Ministry of Social Affairs and Health. The study was performed in accordance with the Helsinki Declaration. Patient data was handled with confidentiality, and according to the instructions of all participating hospitals and laboratories.

5.5 STATISTICS

Data concerning the pregnancies screened in the first trimester were registered, and the parameters of variables were tabulated. It was determined which pregnancies had been tested using AFS or CVS, and the results of the chromosome determinations were collected. On the basis of the screening results and chromosome determinations, the FPR for each group was collected, and the DR in each group was calculated. The DR for each age group (<20, 20–24, 25–29, 30–34, 35–39, 40–44, and ≥45 years) was calculated as the ratio of true positive cases detected in screening divided by the total number of Down's syndrome cases.

The differences between the subject and reference groups were tested for statistical significance. In studies I, II, and IV, the median MOMs for fβ-h and PAPP-A concentration and for the NTs of different study groups were compared using two-tailed pooled t-tests, since the MOMs fitted Gaussian distributions. Continuous variables (mean maternal age, mean weight gain during pregnancy, mean birth weight, the mean duration of the pregnancy) were compared using Student's two-way t-tests. Chi-square tests were used to analyze dichotomous variables (yes/no responses regarding FPR and DR, chronic diseases – hypertension and diabetes, gestational diseases – diabetes and hepatosis, smoking during pregnancy, prior infertility treatment, prior miscarriages, prior induced abortions, prior foetal death, maternal obesity, primigravida, placenta praevia, adverse outcome, caesarean section). The odds ratios for adverse outcomes at different serum marker MOM levels were calculated. Fisher's exact test was used when there were fewer than five units in any of the classes. In study III, the adjusted R-square with p-value of F-test was used to evaluate the goodness of fit in non-linear regression analysis. When P

values were less than 0.05, differences were considered statistically significant. Data in studies I, II, and IV were analyzed using the SAS software package (SAS Institute Inc., Cary, NC, USA). In study III, data analyses were conducted using the SPSS software package (SPSS Inc., Chicago, IL, USA).

6 Results

6.1 THE TIME TO PREGNANCY IN EUPLOID PREGNANCIES AFFECTS THE COMBINED FIRST TRIMESTER SCREENING MARKERS AND RESULTS

A prolonged TTP of ≥25 months was associated with a higher mean maternal age (p<0.01), obesity (p<0.01), chronic diseases (p=0.04), and prior miscarriages (p<0.01). However, despite the clustering of maternal risk factors in the group with prolonged TTP, no statistically significant differences were found in terms of pregnancy duration, mean birth weight, or PA, PD, and PE. These risks and adverse outcomes were associated with IVF pregnancies (p≤0.02), as was the incidence of LBW, even though the prevalence of maternal risk factors in the IVF group was closer to those with no delay in getting pregnant than those with TTP values of ≥13 months.

The median MOM for PAPP-A was lower in the groups with TTP values ≥25 months and that had undergone IVF as compared to the reference group with short TTP (p<0.01 in both groups). No statistically significant differences were found in the median MOMs for fβ-hCG and NT between the groups. The study group with the longest TTP (≥25 months) had the highest FPR (p<0.01). The proportion of SGA in the study population was 9.4 percent. The median PAPP-A concentration for all the SGA pregnancies investigated was 0.85 MOM, whereas for pregnancies resulting in infants with normal birth weights, the PAPP-A MOM was 0.98 MOM (p<0.01). The combined FTS results according to TTP are presented in Table 3.

Table 3. First trimester Down's syndrome screening results for the study groups compared to a reference group for which the time to pregnancy was 0–12 months (N=1385)

Time to pregnancy	No infertility treatment					Assisted reproduction	
	0–12 months N=1164	13–24 months N=112	P	≥25 months N=70	P	IVF N=39	P
Median fβ-hCG MOM	0.97	1.05	0.14	0.94	0.73	0.91	0.80
Median PAPP-A MOM	1.03	1.03	0.91	0.83	<0.01	0.84	<0.01
Median NT MOM	0.98	0.96	0.61	0.94	0.39	1.02	0.19
FPR (%)	2.1	2.7	0.66	12.9	<0.01	2.6	0.76

6.2 LOW PAPP-A IS A MARKER FOR ADVERSE PREGNANCY OUTCOMES WITH PATHOLOGY OF THE PLACENTA

Maternal hypertension was associated with all adverse outcomes studied: PA, PD, PE, and SGA (p<0.01 in each). Diabetes was most common in women with PE and PD (p<0.001 in both groups). Obesity and gestational diabetes were most common in women with PE (p<0.01 for both). The highest occurrence of placenta praevia was in the PD group (p<0.01). The proportion of primigravidas was significantly higher in the groups with PE and SGA (p<0.001 for both groups).

The mean pregnancy duration was shorter in all study groups as compared to the reference group (p<0.01 in each group). Mean foetal birth weight was lowest in the PA group (p<0.01). The mean placental weight / birth weight ratio expressed as a percentage was higher in all study groups as compared to the reference group; the same was true for Caesarean sections, abnormal Apgar scores, the need for intensive care, and infants with LBW (p<0.01 in each group).

The median MOMs for fβ-hCG and PAPP-A were lower in the PD, PE, and SGA groups than in the reference group (p≤0.02 in each group). However, the median MOMs for fβ-hCG and PAPP-A in the PA and the reference groups appeared to be statistically similar. No statistically significant differences between the groups were found in terms of the median MOMs for NT. The highest FPR was observed in the PD group (p<0.01). The combined FTS results in pregnancies with adverse outcomes are presented in Table 4.

Table 4. First trimester Down's syndrome screening in the PE, PED, SGA and PA study groups compared to the reference group (N=2844)

Characteristic	Normal outcome N=2164	PE N=175	P	PD N=213	P	SGA infants N=275	P	PA N=17	P
Median fβ-hCG MOM	0.99	0.86	0.02	0.92	0.02	0.90	<0.01	0.92	0.62
Median PAPP-A MOM	0.99	0.79	<0.01	0.80	<0.01	0.79	<0.01	1.00	0.76
Median NT MOM	1.05	1.06	0.63	1.06	0.52	1.05	0.75	1.22	0.59
FPR (%)	2.6	5.1	0.06	6.6	<0.01	4.7	0.05	0	1.00*

*When a class included less than 5 variables, Fisher´s exact test was used

For all adverse pregnancy outcomes, the odds ratios of fβ-hCG MOM <1.0 were 1.16–1.76, and the odds ratio of PAPP-A MOM <1.0 1.09–2.06. In pregnancies with PD and SGA, the odds ratio with decreasing PAPP-A MOM increased linearly, whereas in pregnancies with PE, the odds ratios were variably higher at low PAPP-A MOMs. In PA pregnancies, the odds ratios were most variable; 1.09 for PAPP-A MOM <1.0, 0.76 for PAPP-A MOM <0.8, 0.52 for PAPP-A MOM <0.6, and 1.73 for PAPP-A MOM <0.4.

6.3 MORE INVASIVE TESTS ARE NEEDED FOR YOUNG WOMEN TO DETECT A DOWN'S SYNDROME PREGNANCY

The DR for the combined FTS was 81.9 percent. The mean maternal age in the study population was 29.3 years. Using a cut-off value of 1:250, the FPR was 4.3 percent. The number of invasive procedures needed to detect one case of DS in the entire population was 1:22. Using an FPR of five percent, the cut-off was calculated as 1:283, and the overall DR would have improved to 82.4 percent with one additional detected DS pregnancy. However, the increase in FPR from 4.3 to 5.0 percent would mean an additional loss of five healthy fetuses due to the increased number of invasive procedures needed to detect one additional case of DS.

The lowest DR (63.6 percent) and the highest number of invasive tests needed were in the age group of 25–29 years (51 tests needed for each case of DS). The highest DR (92.7 percent) and the lowest number of invasive procedures needed were in the age group of 40–44 years (14 tests needed). Using a fixed five percent FPR for all age blocks did not improve the DRs: only one extra case of DS would have been detected, in the group of women aged between 25 and 29, and so the number of

invasive tests needed to detect one case of DS would have increased from 51 to 87. Moreover, six cases in women aged between 35 and 39 would have been missed, decreasing the DR from 83.3 to 75.0 percent, and 14 cases would have been missed in women aged between 40 and 44, decreasing the DR form 92.7 percent to 58.5 percent. Table 5 compares the performance of the combined FTS at a fixed FPR of five percent and a fixed cut-off limit of 1:250.

Table 5. Comparison of the performance of the combined FTS between fixed FPR of 5 % and fixed cut-off limit of 1:250 in the screened population (N=65600), and the Down's syndrome pregnancies (N=118)

				Cut-off 1:250					FPR 5 %				
Age (yrs)	N (%)	T21 N	T21 (%)	FPR (%)	FP N	TP N	DR (%)	NIP N	Cut-off	FP N	TP N	DR %	NIPN
–19	2.4	0	0	1.9	35				1:485	92			
20–24	17.8	14	7.4	2.3	315	11	78.6	29	1:469	685	11	78.6	62
25–29	34.1	22	11.7	2.7	708	14	63.6	51	1:420	1312	15	68.2	87
30–34	28.8	37	19.7	3.2	709	29	78.4	24	1:370	1108	30	81.1	37
35–39	14.0	72	38.3	9.2	991	60	83.3	17	1:110	539	54	75.0	10
40–44	2.8	41	21.8	24.0	517	38	92.7	14	1:20	107	24	58.5	4
45–	0.1	2	1.1	48.1	37	2	100.0	19	1:25	4	2	100.0	2

FP = false positives
TP = true positives
NIPN=number of invasive procedures needed to detect one T21

6.4 MATERNAL AGE AFFECTS THE MEDIANS OF THE BIOCHEMICAL MARKERS

The median MOM for fβ-hCG across the entire studied population was 0.98. The median MOM was 0.99 in women aged <35 years, whereas in women aged ≥35 years it was 0.94 ($p<0.01$). The median MOM of PAPP-A in the entire screened population was 0.94. In women aged <35 years, the median MOM was 0.92, and in women aged ≥35 years it was 0.95. None of the differences between age groups were statistically significant. In DS pregnancies, the overall median MOM for fβ-hCG was 1.58, and the overall median MOM for PAPP-A was 0.40. No statistically significant differences were found in fβ-hCG or PAPP-A MOMs in DS pregnancies between women aged <35 years and ≥35 years. Across the entire screened population, there was an increasing trend in fβ-hCG MOM values and a decreasing trend in PAPP-A MOM values as gestational age increased. In DS pregnancies, the MOMs of fβ-hCG and PAPP-A varied as gestation progressed in women aged <35 years, but in women aged ≥35 years the median MOM for fβ-hCG increased and the median MOM for PAPP-A decreasing as gestation progressed.

The DR of the serum markers was 66.5 percent. In women <35 years, the serum markers gave a DR of only 38.6 percent, whereas in women of ≥35 years, the biochemistry detected 82.7 percent of DS pregnancies ($p<0.01$). The number of invasive procedures needed to detect one case of DS was thus 19 in women <35 years, and 11 in women aged ≥35 years. When the FPR in both age groups was set at five percent, the DR in women aged <35 years increased to 68.2 percent, and in women aged ≥35 years it decreased to 71.9 percent. This clearly shows that the number of invasive procedures needed to detect one case of Down's syndrome was clearly higher in younger than in older age groups; 39 in younger women vs. 5 in older women. Biochemical marker data for women in the different age groups considered are presented in Table 6.

Table 6. Biochemical marker data for women in different age groups from the screened population (N=76949), and the frequency of Down's syndrome pregnancies (N=221) compared to those for women aged 35–39 years

The screened population														
Age block	–19 years (N=1847)	P	20–24 years (N=13697)	P	25–29 years (N=26240)	P	30–34 years (N=22159)	P	35–39 years (N=10773)	40–44 years (N=2154)	P	45– years (N=79)	P	All (N= 76949)
Median fβ-hCG MOM	1.06	<0.01	1.00	<0.01	0.99	<0.01	0.96	0.06	0.94	0.94	0.30	1.05	0.38	0.98
Median PAPP-A MOM	0.87	<0.01	0.89	<0.01	0.91	0.02	0.95	0.02	0.96	0.91	0.23	0.83	0.81	0.95
FPR (%)	1.1	<0.01	1.7	<0.01	2.4	<0.01	3.0	<0.01	8.6	25.5	<0.01	55.7	<0.01	4.1
Women aged <35 years (N=63943)									Women aged ≥35 years (N=13006)					P
Median fβ-hCG MOM								0.99					0.94	<0.01
Median PAPP-A MOM								0.93					0.96	0.08
FPR (%)								2.4					11.6	<0.01
The Down's syndrome pregnancies														
Age block	–19 years (N=1)	P	20–24 years (N=17)	P	25–29 years (N=22)	P	30–34 years (N=42)	P	35–39 years (N=84)	40–44 years (N=51)	P	45– years (N=4)		All cases (N=221)
Median fβ-hCG MOM	1.27	0.61	1.57	0.89	1.73	0.62	1.51	0.57	1.60	1.71	0.75	1.45		1.58
Median PAPP-A MOM	0.13	0.11	0.78	0.15	0.34	0.20	0.50	0.05	0.40	0.45	0.63	0.40		0.40
DR (%)	100		29.4	<0.01	36.4	<0.01	40.5	<0.01	76.2	90.2	0.57	100		66.5
DR at 5 % FPR		100		70.6		68.2		66.7	71.4		70.6	100		70.6
NIPN		93		41		60		27	7		3	1		18
Women aged <35 years (N=82)									Women aged ≥35 years (N=139)					P
Median fβ-hCG MOM								1.53					1.60	0.92
Median PAPP-A MOM								0.43					0.38	0.31
DR (%)								38.6					82.7	<0.01
DR at 5 % FPR								68.2					71.9	0.38
NIPN								39						5

NIPN = number of invasive tests needed to detect one T21

In the entire screened population, the median gestational age was week 11+0. Therefore, this gestational group was used as a reference group for statistical evaluation. For the reference group of 11 weeks, the median MOM of fβ-hCG was 0.99, and the median MOM of PAPP-A was 0.99. The median fβ-hCG MOM was 0.99–1.06 during pregnancy weeks 8–13, but the median MOM of PAPP-A showed a greater variance depending on the stage of gestation, being <0.70 during pregnancy weeks 8–9, and >0.93 from week 10 onwards. The highest DR for the biochemical markers was achieved at the end of gestational week 9 (96.2 percent), while the lowest detection rate was found at the end of week 11 (56.5 percent) ($p<0.01$). The median maternal age was 38 years for both groups. When the FPR in each age group was set at five percent, the DR was still highest in week 9 (88.5 percent) but lowest in week 8 (58.3 percent). However, when using this fixed five percent cut-off, the number of invasive procedures needed to detect one case of Down's syndrome was highest in week 10 and lowest in weeks 8 and 13 (22 vs. 9). The median MOMs and DRs for selected gestational weeks are presented in Table 7.

Table 7. Median MOMs and detection rates in the screened population (N=76949), and the Down's syndrome pregnancies (N=221), in selected gestational weeks compared to gestational week 11.

Completed weeks	8	P	9	P	10	P	11	12	P	13	P
The screened population											
Median age		29		29		29	29		30		31
N		2013		7906		17048	25440		21662		2880
Median fβ-hCG MOM	1.03	0.47	1.06	<0.01	1.00	0.55	0.99	1.02	<0.01	1.03	0.03
Median PAPP-A MOM	0.48	<0.01	0.68	<0.01	0.95	<0.01	0.99	0.96	<0.01	0.93	<0.01
FPR (%)	11.0	<0.01	8.9	<0.01	3.8	0.35	4.0	3.6	0.06	5.5	0.03
The Down's syndrome pregnancies											
Median age		37		38		35	38		37		32
N		12		26		40	62		65		16
Median fβ-hCG MOM	1.91	0.81	1.69	0.71	1.42	0.44	1.56	1.60	0.92	1.57	0.41
Median PAPP-A MOM	0.27	0.13	0.20	<0.01	0.32	0.03	0.49	0.47	0.84	0.44	0.59
DR (%)	75.0	0.34	96.2	<0.01	67.5	0.26	56.5	63.1	0.45	64.7	0.54
DR at 5 % FPR		58.3		88.5		72.5	59.0		65.6		58.9
NIPN		9		16		22	21		17		9

NIPN = number of invasive tests needed to detect one T21

7 Discussion

7.1 SUMMARY OF THE MAIN FINDINGS

In study I, the combined FTS markers were compared between spontaneous pregnancies with different TTPs to those in IVF pregnancies. We found that a prolonged TTP is, at some level, associated with an increased FPR in the combined FTS. This raises a question of whether there is a need to adjust PAPP-A in the risk calculation for the waiting time to pregnancy. The observed difference in PAPP-A concentration is likely to have contributed in concert with maternal age to the calculation of DS risk, and further studies are needed before considering adjustments in the risk calculations for TTP, as well as for maternal age.

In study II, we investigated the profile of the biochemical markers in adverse pregnancy outcomes, including PA, in chromosomally normal pregnancies. The mechanism involved with the most adverse outcomes such as PD, PE, and SGA may be related to reduction in the protease activity of PAPP-A which affects the concentration of free IGF in early pregnancy. Low fβ-hCG and PAPP-A levels predict placental diseases – PE, PD, and SGA –, but not decidual diseases such as PA.

In studies III–IV, the DRs and the numbers of invasive procedures needed to detect one DS pregnancy in women between 15 and 48 years of age were examined according to 5-year age groupings. In addition, the performance of the first trimester biochemical screening markers at different maternal ages was analysed. The results reminded that it is absolutely crucial for all women participating in the prenatal screening program to be informed of the shortcomings of the available screening methods with regard to the maternal age. Fβ-hCG and PAPP-A are of limited utility for detecting DS pregnancies in women under the age of 35, who represent the majority of pregnant women. Therefore, in young women, combined screening should be the method of choice. Since there was considerable variation in the levels of the biochemical markers, and especially PAPP-A, between the different age groups examined, we suggest that further research should focus on determining whether there is a need to introduce age-related MOMs in DS screening.

7.2 THE VALIDITY OF THE RESULTS

The key aim of the work described in this doctoral thesis was to examine the quality of the combined FTS. The study data was gathered using a routine screening method and therefore, the screened population provided an accurate representation of the entire pregnant population. More than 70 percent of pregnant women entered the combined FTS during the study period (Marttala et al. 2011, III). Extensive maternal information was gathered during pregnancy and the information was routinely registered with the maternity care units. The hospital-based registries of births and the national registers are reliable and extensive (Teperi 1993). The combined FTS was carried out under continuous monitoring during the pre-analytical, analytical, and post-analytical phases of blood sample collection. Invasive tests were performed by specialists, and the chromosome analyses were conducted at major accredited laboratories. In addition, internal and external quality controls were used in the risk calculation program and the laboratory methods for the biochemical markers in the combined FTS. All these factors suggest that the results for the normal screened population are valid. However, the incidence of DS pregnancies and adverse

pregnancy outcomes is probably underestimated, due to spontaneous abortion. Presumably not all DS pregnancies in the study population were found. The diagnoses of adverse outcomes, especially the recessive cases of PA, may be incomplete since these conditions have few symptoms.

The screening during pregnancy is much more extensive than mere DS screening. For instance, it involves collecting and verifying data influencing the pregnancy; twinning is diagnosed, the expected date of delivery is verified, and other chromosomal disorders are screened. These examinations rely on ultrasound scans that are properly performed. Unfortunately, when the screening data were collected, there were no regulations in Finland with regard to quality control or auditing of the NT or other first trimester ultrasonic measurements, and no training program was available to the midwives or gynaecologists performing the NT measurements. It is therefore possible that the NT measurements may have been mis-estimated, which would have influenced the observed DR values. The DRs and the data for the biochemical and the combined FTS are presented and compared to previously published results in Table 8. Some large studies, with a similar population size to ours, have reported higher DRs than found in the present study (Nicolaides et al. 2005). This is probably a result of major centralising and adequate calibration of the MOMs, as well as ideal timing of blood sampling and the NT measurement (Wright et al. 2010).

Table 8. The performance of screening and the incidence of invasive procedures using different FTS methods.

				fβ-hCG and PAPP-A			Combined FTS		
Study	N	Mean age (yrs)	Incidence of DS (%)	FPR (%)	DR (%)	NIPN	FPR (%)	DR (%)	NIPN
Malone et al. 2005	36120	27.1	0.25				5.0	87	23
Wald et al. 2003	47053	29	0.21	5.0	83	24	5.0	93	23
Marttala et al. 2011 (III), Ranta et al. 2011 (IV)	**76949**	**29.3**	**0.29**	**4.1**	**67**	**18**	**4.3**	**82**	**22**
Crossley et al. 2002	17229	29.9	0.26	5.0	55	37	5.0	82	30
Spencer et al. 2003	12339	30	0.20				5.2	92	35
Nicolaides et al. 2005	75821	31	0.43	5.0	67	12	5.0	90	13
Krantz et al. 2000	10251	31.8	0.49				5.0	91	11
Orlandi et al. 1997	2010	32	0.55	5.0	61	10	5.0	87	10
Bindra et al. 2002	14383	34	0.57	5.0	60	15	5.0	90	10
Wapner et al. 2003	8514	34	0.72	5.0	67	10	5.0	79	9
Kagan et al. 2009	19736	34.5	0.62				5.0	93	9

In studies I–II, there was a problem with the small size of the screened population, with the PA and IVF groups being particularly small. These results should be confirmed in a larger study. In addition, the reliability of the TTP data obtained in

study I could be questioned. TTP is anamnestic information, and is not solely dependent on the mother, but depending also on the factors associated with the male partner to the pregnant woman.

During the study, an error in the risk calculation was found in the median MOM of PAPP-A. In the entire screened population when the results of both Oulu and Kuopio University Hospital catchment areas were combined, the median MOM of PAPP-A was <1.00, indicating that the medians in the risk calculation programme calibration curve were not adequately fitted. Especially for women aged <25 years, who had the PAPP-A median MOM <0.90, the median MOMs of PAPP-A should be specified in following versions of the risk calculation programme, probably by collecting normal values from a larger population. The medians for this specific version of the risk calculation programme were collected from samples in a pregnant population at the mean maternal age of 29 years, and therefore this age group was over-presented. In addition, the distribution of external quality control samples represented a childbearing population with a similar age distribution.

7.3 CLINICAL SIGNIFICANCE AND APPLICATION OF THE RESULTS

The criteria for screening established by the WHO (WHO 1968) do not seem to be met completely in existing screens for DS or adverse pregnancy outcomes. DS and most adverse outcomes are not major health problems, and their incidence at the population level is low. Only PD has an incidence of more than five percent. There are no suitable tests with sufficient accuracy to screen for adverse outcomes in low-risk populations. No established screening method exists to screen for most of the adverse pregnancy outcomes, including PA and PD. As the majority of adverse pregnancy outcomes are multifactorial, there is currently no evidence that routine screening for any of these conditions would significantly improve the outcome. In addition, no efficient intervention besides emphasized monitoring is available, and its benefits remain to be proven. Thus the costs of screening for adverse outcomes cannot be estimated. However, in addition to DS, there is a clinically recognizable stage during pregnancy for PE, and a suitable screening test for recognition, an agreement of whom to treat as patients (American Congress of Obstetricians and Gynecologists 2002), and case finding is a continuous process. Furthermore, clinical evidence-based intervention protocols are available (Bujold et al. 2009, WHO 2011).

Even though pregnancies with moderate or high risks for developing PD, PE or SGA can be recognized during the first trimester by their low concentrations of fβ-hCG and PAPP-A, the incidence of PA appeared to be unpredictable on the basis of the combined FTS markers even in cases where patient and familial history both flagged up patients at risk. In addition, while the lower than normal concentrations of fβ-hCG and PAPP-A were statistically significant in PD, PE, and SGA pregnancies, the MOMs for fβ-hCG in each group still were within 10 percent of the normal values. Only the PAPP-A concentrations were ≥20 percent lower in pregnancies involving PE, PD and SGA, whereas in DS pregnancies the concentrations are 50 percent lower. However, it seems that the way of thinking is changing in screening of adverse pregnancy outcomes and maternal risk factors should be recognised at the earliest possible stage (Nicolaides 2011). Changes in concentrations may have an effect on the results of screening, when maternal risk factors – amongst others smoking, very high maternal age, or TTP of over two years – are recognised during the first trimester (Nicolaides 2011). In adverse outcomes, if susceptibility for developing PE and SGA, perhaps PD, can be identified, more resources could be guided towards more efficient follow-up and health support in these women at risk.

If adverse outcomes are wanted screened, a new algorithm for this kind of risk assessment should to be designed.

Subfertile women, defined as those with TTP of two years or more, had significantly reduced serum PAPP-A concentrations in comparison to those showing normal fertility. Along with higher maternal age, the lower concentrations of PAPP-A led to a higher FPR in DS screening. A high maternal age and a prolonged TTP were found to increase the risk of an adverse outcome. The risk calculation program does not factor in risks that may be presented by high maternal age, such as chronic diseases. Maternal age affects the median PAPP-A concentration but the MOMs are not age-adjusted – only the pregnancy-specific DS risk ratio is age-adjusted. More research into the effect of age on MOMs will be needed before any attempt at introducing such age adjustments.

The main difference between IVF women and those who took a long time to achieve a spontaneous pregnancy was the application of assisted reproduction techniques. Therefore, the lower than normal concentration of PAPP-A may also have affected the screening results for IVF pregnancies. FTS can equally well be applied to IVF pregnancies although the origin of the pregnancy should be noted.

Using a fixed FPR of five percent would increase the DR only in women over 24 years of age, or only after gestational week 10. The most problematic age group in the screening proved to be women aged 25–29 years, who actually represented the most numerous group of the screened population. The manipulation of the cut-off level or FPR in the screening program would not lead to an improvement of the DR in young women, but possibly it would improve the DR in early first trimester screening. In the combined FTS, if both the biochemical markers and the NT scan are interpreted as normal in women aged ≥35 years, the number of invasive procedures could be reduced, as the majority of false negative screening results occur in women aged <35 years. If, however, a high NT indicates an elevated risk, the results of the biochemical screening are less important, especially in younger women. The effect of maternal and gestational ages should to be considered when examining the results of the combined FTS, both in euploid and aneuploid pregnancies. Patients should be counselled with regard to their age. In addition, the timing of the blood sampling at weeks 9–10 seems justified.

7.4 FUTURE CONSIDERATIONS FOR THE COMBINED FIRST TRIMESTER SCREENING

Methods of screening for DS during the first trimester are continuously under examination, worldwide. New markers are tested to assess their suitability for routine use; markers currently undergoing this process include ultrasonic venous duct flow changes (Matias et al. 1999) or the absence of the nasal bone (Cicero et al 2001). The concentration of a disintegrin and metalloproteinase 12 (ADAM12) during the first trimester have been reported to be reduced in DS pregnancies (Laigaard et al. 2006, Valinen et al. 2009, Koster et al. 2011), and therefore ADAM12 has been suggested as a possible fourth first trimester screening marker. Foetal DNA and especially the extent of its methylation, measured from the maternal serum, can be studied to diagnose DS non-invasively (Chim et al. 2008, Papageorgiou et al. 2011). If this was feasible, screening would naturally step aside as the diagnosis would be achieved at around the time that screening is performed at present.

To date, new markers reported in the academic literature have not yet entered into routine use and for the time being, the focus is on developing the quality of the combined FTS. The studies reported in this thesis have demonstrated that the

performance of combined FTS is reasonably good but it can be improved. This has been acknowledged by staff at the Eastern Finland Laboratory Centre and Kuopio University Hospital, which was one of the first Finnish institutions to offer the combined FTS in 2002. The risk calculation program is used routinely to calculate the risk ratio for trisomy 18, in addition to DS. The screening as a whole is well-audited. Quality control of the NT measurements is performed continuously: the personnel performing the measurements perform approximately 500 repetitions yearly, which is markedly more than the nationally recommended minimum, and a Performer-specific follow-up in NT measurements has been in use since 2011 in the maternity clinic of Kuopio University Hospital. The study cannot compare the results in the performance of the NT measurements, but the quality control and training of the personnel involved in performing the NT measurements should lead to improvement in DRs for all age groups. In order to improve the utility of the combined FTS markers, the focus is on MOM-based assessment of the results and risk calculation. Centralising the NT measurements, the blood sample collection and their analyses, as well as risk calculation in major accredited laboratories also improves the quality of the combined FTS. Centralising means more repetitions to the health care personnel working with the combined FTS, constructing routines, and reducing the probability of mistakes. By centralizing the first trimester ultrasound scans, the measurement of CRL becomes more accurate and thus the estimation of NT more precise, which also improves the performance of the screening (Kagan et al. 2012).

References

Aitken DA, McCaw G, Crossley JA, Berry E, Connor JM, Spencer K, Macri JN. First-trimester biochemical screening for fetal chromosome abnormalities and neural tube defects. Prenat Diagn. 1993 Aug;13(8):681-9.

Aitken DA, Wallace EM, Crossley JA, Swanston IA, van Pareren Y, van Maarle M, Groome NP, Macri JN, Connor JM. Dimeric inhibin A as a marker for Down's syndrome in early pregnancy. N Engl J Med. 1996 May 9;334(19):1231-6.

Alfthan H, Schröder J, Fraser R, Koskimies A, Halila H, Stenman UH. Choriogonadotropin and its beta subunit separated by hydrophobic-interaction chromatography and quantified in serum during pregnancy by time-resolved immunofluorometric assays. Clin Chem. 1988 Sep;34(9):1758-62.

Altmann P, Kucera H. [Influence of age on risk-factors during pregnancy, delivery and puerperium of primiparae (author's transl)]. [Article in German] Geburtshilfe Frauenheilkd. 1975 Mar;35(3):218-24.

American Congress of Obstetricians and Gynecologists practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33. *Obstet Gynecol.* 2002 january;99:159–167.

Amor DJ, Xu JX, Halliday JL, Francis I, Healy DL, Breheny S, Baker HW, Jaques AM. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome. Hum Reprod. 2009 Feb 26.

Ananth CV, Wilcox AJ. Placental abruption and perinatal mortality in the United States. Am J Epidemiol. 2001 Feb 15;153(4):332-7.

Anckaert E, Schiettecatte J, Sleurs E, Devroey P, Smitz J. First trimester screening for Down's syndrome after assisted reproductive technology: non-male factor infertility is associated with elevated free beta-human chorionic gonadotropin levels at 10-14 weeks of gestation. Fertil Steril. 2008 Oct;90(4):1206-10.

Andersch B, Svensson A, Hansson L. Characteristics of hypertension in pregnancy. A retrospective study of 261 consecutive cases. Acta Obstet Gynecol Scand Suppl. 1984;118:33-8.

Asmala K. Downin oireyhtymän seulonta Suomessa. Suom Lääkäril 50(25): 2585-88, 1995.

Audibert F, Gagnon A. Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada; Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists. Prenatal screening for and diagnosis of aneuploidy in twin pregnancies. J Obstet Gynaecol Can. 2011 Jul;33(7):754-67.

Autti-Rämö I, Koskinen H, Mäkelä M, Ritvanen A, Taipale P and working group. Maternal ultrasound and serum screening in the detection of structural and chromosomal abnormalities. Finnish Office for Health Technology Assessment FinOHTA/National Research and Development Centre for Welfare and Health/STAKES. Helsinki, Finland 2005. ISBN 951-33-1796-X. ISSN 1239-6273. Available at: http://finohta.stakes.fi/NR/rdonlyres/63D73A8E-E1CF-4922-BD65-01D61F6C490E/0/r027f.pdf. Accessed Feb 27, 2012.

Bale LK, Conover CA. Disruption of insulin-like growth factor-II imprinting during embryonic development rescues the dwarf phenotype of mice null for pregnancy-associated plasma protein-A. J Endocrinol. 2005 Aug;186(2):325-31.

Beaman JM, Goldie DJ. Second trimester screening for Down's syndrome: 7 years experience. J Med Screen. 2001;8(3):128-31.

Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, Rubens C, Menon R, Van Look PF. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ. 2010 Jan;88(1):31-8. Epub 2009 Sep 25.

Bérard A, Le Tiec M, De Vera MA. Study of the costs and morbidities of late-preterm birth. Arch Dis Child Fetal Neonatal Ed. 2012 Jan 31. [Epub ahead of print]

Bernaschek G, Yildiz A, Kolankaya A, Stuempflen I, Deutinger J. Complications of cordo- centesis in high-risk pregnancies: effects on fetal loss or preterm delivery. Prenat Diagn
1995;15(11):995-1000.

Bersinger NA, Klopper A. Serum concentration of pregnancy-associated plasma protein A in the first trimester of pregnancy. Am J Obstet Gynecol. 1984 Nov 15;150(6):780-2.

Bewley S, Roberts LJ, Mackinson AM, Rodeck CH. First trimester fetal nuchal translucency: problems with screening the general population. 2. Br J Obstet Gynaecol. 1995 May;102(5):386-8.

Bielanska M, Tan SL, Ao A. Chromosomal mosaicism throughout human preimplantation development in vitro: incidence, type, and relevance to embryo outcome. Hum Reprod. 2002 Feb;17(2):413-9.

Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. One-stop clinic for assessment of risk for trisomy 21 at 11-14 weeks: a prospective study of 15 030 pregnancies. Ultrasound Obstet Gynecol. 2002 Sep;20(3):219-25.

Bliumina MG, Lil'in ET, Patiutko RS. [Multiple birth and Down's disease]. [Article in Russian] Genetika. 1975;11(1):153-4.

Bodnar LM, Catov JM, Zmuda JM, Cooper ME, Parrott MS, Roberts JM, Marazita ML, Simhan HN. Maternal serum 25-hydroxyvitamin D concentrations are

associated with small-for-gestational age births in white women. J Nutr. 2010 May;140(5):999-1006. Epub 2010 Mar 3.

Bogart M, Pandian M, Jones O. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat Diagn 1987;7:623-30.

Brambati B, Macintosh MC, Teisner B, Maguiness S, Shrimanker K, Lanzani A, Bonacchi I, Tului L, Chard T, Grudzinskas JG. Low maternal serum level of pregnancy associated plasma protein (PAPP-A) in the first trimester in association with abnormal fetal karyotype. Br J Obstet Gynaecol 1993;100:324-6.

Brambati B, Tului L, Bonacchi I, Shrimanker K, Suzuki Y, Grudzinskas JG. Serum PAPP-A and free beta-hCG are first-trimester screening markers for Down syndrome. Prenat Diagn 1994;14(11):1043-7.

Brameld KJ, Dickinson JE, O'Leary P, Bower C, Goldblatt J, Hewitt B, Murch A, Stock R. First trimester predictors of adverse pregnancy outcomes. Aust N Z J Obstet Gynaecol. 2008 Dec;48(6):529-35.

Breathnach FM, Malone FD, Lambert-Messerlian G, Cuckle HS, Porter TF, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, Klugman S, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Tripp T, Bianchi DW, D'Alton ME. First and Second Trimester Evaluation of Risk (FASTER) Research Consortium. First- and second-trimester screening: detection of aneuploidies other than Down syndrome. Obstet Gynecol. 2007 Sep;110(3):651-7.

Buchin PJ, Levy JS, Schullinger JN. Down's syndrome and the gastrointestinal tract. J Clin Gastroenterol. 1986 Apr;8(2):111-4.

Bujold E, Morency AM, Roberge S, Lacasse Y, Forest JC, Giguère Y. Acetylsalicylic acid for the prevention of preeclampsia and intra-uterine growth restriction in women with abnormal uterine artery Doppler: a systematic review and meta-analysis. J Obstet Gynaecol Can. 2009 Sep;31(9):818-26.

Canick J, Knight G, Palomaki G, Haddow J, Cuckle H, Wald N. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. Br J Obstet Gynaecol 1988;95:330-3.

Catov JM, Nohr EA, Olsen J, Ness RB. Chronic hypertension related to risk for preterm and term small for gestational age births. Obstet Gynecol. 2008 Aug;112(2 Pt 1):290-6.

Chemnitz J, Tornehave D, Teisner B, Poulsen HK, Westergaard JG. The localization of pregnancy proteins (hPL, SP1 and PAPP-A) in intra- and extrauterine pregnancies. Placenta. 1984 Nov-Dec;5(6):489-94.

Chim SS, Jin S, Lee TY, Lun FM, Lee WS, Chan LY, Jin Y, Yang N, Tong YK, Leung TY, Lau TK, Ding C, Chiu RW, Lo YM. Systematic search for placental DNA-methylation markers on chromosome 21: toward a maternal plasma-based epigenetic test for fetal trisomy 21. Clin Chem. 2008 Mar;54(3):500-11. Epub 2008 Jan 17.

Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with trisomy 21 at 11-14 weeks of gestation: an observational study. Lancet. 2001 Nov 17;358(9294):1665-7.

Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, Lammoglia R, Charnock-Jones DS. A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. Biol Reprod. 1998 Dec;59(6):1540-8.

Collacott RA, Cooper SA, McGrother C. Differential rates of psychiatric disorders in adults with Down's syndrome compared with other mentally handicapped adults. Br J Psychiatry 1992;161:671-4.

Coskun A, Balbay O, Duran S, Annakkaya AN, Bulut I, Yavuz O, Kurt E. Pregnancy-associated plasma protein-A and asthma. Adv Ther. 2007 Mar-Apr;24(2):362-7.

Crossley JA, Aitken DA, Cameron AD, McBride E, Connor JM. Combined ultrasound and biochemical screening for Down's syndrome in the first trimester: a Scottish multicentre study. BJOG. 2002 Jun;109(6):667-76.

Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. Lancet 1984;1(8383):926-9.

Cuckle H, Lilford RJ, Teisner B, Holding S, Chard T, Grudzinskas JG. Pregnancy associated plasma protein A in Down's syndrome. BMJ. 1992 Aug 15;305(6850):425.

Cuckle HS, van Lith JM. Appropriate biochemical parameters in first-trimester screening for Down syndrome. Prenat Diagn. 1999 Jun;19(6):505-12.

Cuckle H. Biochemical screening for Down syndrome. Eur J Obstet Gynecol Reprod Biol. 2000 Sep;92(1):97-101.

Cuevas KD, Silver DR, Brooten D, Youngblut JM, Bobo CM. The cost of prematurity: hospital charges at birth and frequency of rehospitalizations and acute care visits over the first year of life: a comparison by gestational age and birth weight. Am J Nurs. 2005 Jul;105(7):56-64; quiz 65.

Czeizel AE, Bánhidy F. Chronic hypertension in pregnancy. Curr Opin Obstet Gynecol. 2011 Apr;23(2):76-81.

de Graaf IM, Pajkrt E, Bilardo CM, Leschot NJ, Cuckle HS, van Lith JM. Early pregnancy screening for fetal aneuploidy with serum markers and nuchal translucency. Prenat Diagn. 1999 May;19(5):458-62.

Desgualdo CM, Riera R, Zucchi P. Cost estimate of hospital stays for premature newborns in a public tertiary hospital in Brazil. Clinics (Sao Paulo). 2011;66(10):1773-7.

Diderholm B. Perinatal energy metabolism with reference to IUGR & SGA: studies in pregnant women & newborn infants. Indian J Med Res. 2009 Nov;130(5):612-7.

Dolea C, AbouZhar C. Global burden of hypertensive disorders of pregnancy in the year 2000. Evidence and Information for Policy (EIP), World Health Organization, Geneva, July 2003. Global burden of Disease 2000. Available at: http://www.who.int/healthinfo/
statistics/bod_hypertensivedisordersofpregnancy.pdf. Accessed Feb 27, 2012.

Dommisse J, Tiltman AJ. Placental bed biopsies in placental abruption. Br J Obstet Gynaecol. 1992 Aug;99(8):651-4.

Down JLH. Observations on an ethnic classification of idiots. Clinical Lecture Reports, 1866, London Hospital 3: 259–262. Available at http://www.neonatology.org/classics/down.html. Accessed Dec 11, 2011.

Dugoff L, Hobbins JC, Malone FD, Porter TF, Luthy D, Comstock CH, Hankins G, Berkowitz RL, Merkatz I, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Vidaver J, D'Alton ME. First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). Am J Obstet Gynecol. 2004 Oct;191(4):1446-51.

Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol. 2009 Jun;33(3):130-7.

Favilli A, Pericoli S, Acanfora MM, Bini V, Di Renzo GC, Gerli S. Pregnancy outcome in women aged 40 years or more. J Matern Fetal Neonatal Med. 2011 Nov 29. [Epub ahead of print]

Finley WH. Effect of drugs on chromosome structure. Am J Clin Nutr. 1975 May;28(5):521-9.

Finnish acts and decrees. Law on pregnancy abortion (In Finnish) 1970. Available at: http://www.finlex.fi/fi/laki/ajantasa/1970/19700239. Accessed Feb 2, 2012.

Finnish acts and decrees. National Health Law. 1972 (In Finnish). Available at: http://www.finlex.fi/fi/laki/ajantasa/1972/19720066. Accessed Feb 27, 2012.

Finnish Government Decree on Screenings. 2006 (In Finnish). Available at: http://www.finlex.fi/fi/laki/alkup/2006/20061339. Accessed Feb 27, 2012.

Finnish Government Decree on Screenings. 2009 (In Finnish). Available at: http://www.finlex.fi/fi/laki/alkup/2009/20090280. Accessed Feb 27, 2012.

Finnish Ministry of Social Welfare and Health. The group of specialists memorandum. 2009 (In Finnish). Available at: http://www.thl.fi/thl-client/pdfs/8a86864a-93a3-426b-ba2d-64742b8cbc28. Accessed Feb 27, 2012.

Galbally M, Snellen M, Lewis AJ. A review of the use of psychotropic medication in pregnancy. Curr Opin Obstet Gynecol. 2011 Dec;23(6):408-14.

Ghisoni L, Ferrazi E, Castagna C, Levi Setti PE, Masini AC, Pigni A. Prenatal diagnosis after ART success: the role of early combined screening tests in councelling pregnant patients. Placenta. 2003 Oct;24 Suppl B:S99-S103.

Gilbert RE, Augood C, Gupta R, Ades AE, Logan S, Sculpher M, van Der Meulen JH. Screening for Down's syndrome: effects, safety, and cost effectiveness of first and second trimester strategies. BMJ. 2001 Aug 25;323(7310):423-5.

Gjerris AC, Loft A, Pinborg A, Christiansen M, Tabor A. First-trimester screening markers are altered in pregnancies conceived after IVF/ICSI. Ultrasound Obstet Gynecol. 2009 Jan;33(1):8-17.

Grenier A, Morissette J, Valet JP, Bélanger L. Polystyrene tube immunoradiometric assay for human alpha1-fetoprotein, and its use for mass screening. Clin Chem. 1978 Dec;24(12):2158-60.

Haddow JE, Palomaki GE, Knight GJ, Canick JA, Wald NJ, Cuckle HS. Maternal serum unconjugated estriol levels are lower in the presence of fetal Down syndrome. Am J Obstet Gynecol. 1990 Oct;163(4 Pt 1):1372-4.

Haddow JE, Palomaki GE, Knight GJ, Williams J, Pulkkinen A, Canick JA, Saller DN Jr, Bowers GB. Prenatal screening for Down's syndrome with use of maternal serum markers. N Engl J Med 1992;327:588-93.

Hafner E, Schuchter K, Philipp K. Screening for chromosomal abnormalities in an unselected population by fetal nuchal translucency. Ultrasound Obstet Gynecol. 1995 Nov;6(5):330-3.

Hagberg B, Kyllerman M. Epidemiology of mental retardation--a Swedish survey. Brain Dev. 1983;5(5):441-9.

Harlap S, Davies AM. Late sequelae of induced abortion: complications and outcome of pregnancy and labor. Am J Epidemiol. 1975 Sep;102(3):217-24.

Heinonen S, Ryynänen M, Kirkinen P, Penttilä I, Syrjänen K, Seppälä M, Saarikoski S. Prenatal screening for congenital nephrosis in east Finland: results and impact on the birth prevalence of the disease. Prenat Diagn. 1996 Mar;16(3):207-13. (a)

Heinonen S, Ryynänen M, Kirkinen P, Hippeläinen M, Saarikoski S. Effect of in vitro fertilization on human chorionic gonadotropin serum concentrations and Down's syndrome screening. Fertil Steril. 1996 Sep;66(3):398-403. (b)

Hiersche HD, Prillwitz S, Tietze KW, Miller R. [Pregnancy in teenagers (author's transl)]. [Article in German] Geburtshilfe Frauenheilkd. 1975 Feb;35(2):112-21.

Horta BL, Victora CG, Menezes AM, Halpern R, Barros FC. Low birthweight, preterm births and intrauterine growth retardation in relation to maternal smoking. Paediatr Perinat Epidemiol. 1997 Apr;11(2):140-51.

Hui PW, Lee CP, Tang MH, Ho PC. Nuchal translucency in pregnancies conceived after assisted reproduction technology. Curr Opin Obstet Gynacol. 2006 Jun;18(3):319-24.

Hyett J, Moscoso G, Papapanagiotou G, Perdu M, Nicolaides KH. Abnormalities of the heart and great arteries in chromosomally normal fetuses with increased nuchal translucency thickness at 11-13 weeks of gestation. Ultrasound Obstet Gynecol. 1996 Apr;7(4):245-50.

Hyett J, Noble P, Sebire NJ, Snijders R, Nicolaides KH. Lethal congenital arthrogryposis presents with increased nuchal translucency at 10-14 weeks of gestation. Ultrasound Obstet Gynecol. 1997 May;9(5):310-3.

Iversen KK, Teisner B, Winkel P, Gluud C, Kjøller E, Kolmos HJ, Hildebrandt PR, Hilden J, Kastrup J; CLARICOR Trial Group. Pregnancy associated plasma protein-A as a marker for myocardial infarction and death in patients with stable coronary artery disease: a prognostic study within the CLARICOR Trial. Atherosclerosis. 2011 Jan;214(1):203-8. Epub 2010 Nov 3.

James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, Yi P, Tafoya DL, Swenson DH, Wilson VL, Gaylor DW. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr. 1999 Oct;70(4):495-501.

Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free beta-hCG and pregnancy-associated plasma protein-A. Hum Reprod. 2008 Sep;23(9):1968-75. Epub 2008 Jun 10.

Kagan KO, Etchegaray A, Zhou Y, Wright D, Nicolaides KH. Prospective validation of first-trimester combined screening for trisomy 21. Ultrasound Obstet Gynecol. 2009 Jul;34(1):14-8.

Kagan K, Hoopmann M, Baker A, Huebner M, Abele H, Wright D. The impact of bias in the crown rump length measurement on first trimester screening for trisomy 21. Ultrasound Obstet Gynecol. 2012 Jan 20. doi: 10.1002/uog.11095. [Epub ahead of print]

Khalil A, Pajkrt E, Chitty LS. Early prenatal diagnosis of skeletal anomalies. Prenat Diagn. 2011 Jan;31(1):115-24. doi: 10.1002/pd.2676.

Kjerulff LE, Sanchez-Ramos L, Duffy D. Pregnancy outcomes in women with polycystic ovary syndrome: a metaanalysis. Am J Obstet Gynecol. 2011 Jun;204(6):558.e1-6. Epub 2011 Mar 16.

Klein EA. Tumor markers in testis cancer. Urol Clin North Am. 1993 Feb;20(1):67-73.

Koivisto M, Jouppila P, Kauppila A, Moilanen I, Ylikorkala O. Twin pregnancy. Neonatal morbidity and mortality. Acta Obstet Gynecol Scand Suppl. 1975;44:21-9.

Koster MP, Wortelboer EJ, Stoutenbeek P, Visser GH, Schielen PC. Modeling the Down syndrome screening performance using first trimester serum markers. Ultrasound Obstet Gynecol. 2011 Aug;38(2):134-9. doi: 10.1002/uog.8881. Epub 2010 Nov 12.

Krantz DA, Larsen JW, Buchanan PD, Macri JN. First-trimester Down syndrome screening: free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. Am J Obstet Gynecol. 1996 Feb;174(2):612-6.

Krantz DA, Hallahan TW, Orlandi F, Buchanan P, Larsen JW Jr, Macri JN. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. Obstet Gynecol. 2000 Aug;96(2):207-13.

Kåregård M, Gennser G. Incidence and recurrence rate of abruptio placentae in Sweden. Obstet Gynecol. 1986 Apr;67(4):523-8.

Lai F, Williams RS. A prospective study of Alzheimer disease in Down syndrome. Arch Neurol 1989;46:849-5.

Laigaard J, Spencer K, Christiansen M, Cowans NJ, Larsen SO, Pedersen BN, Wewer UM. ADAM 12 as a first-trimester maternal serum marker in screening for Down syndrome. Prenat Diagn. 2006 Oct;26(10):973-9.

Lambert-Messerlian GM, Eklund EE, Malone FD, Palomaki GE, Canick JA, D'Alton ME. Stability of first- and second-trimester serum markers after storage and shipment. Prenat Diagn. 2006;26:17-21.

Lambert-Messerlian G, Palomaki GE, Canick JA. Adjustment of serum markers in first trimester screening. J Med Screen. 2009;16(2):102-3.

Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, Yates JR 3rd, Conover CA. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. Proc Natl Acad Sci U S A. 1999 Mar 16;96(6):3149-53.

Lejeune, J; Gautier, M; Turpin, R (1959). "Etude des chromosomes somatiques de neuf enfants mongoliens". Comptes Rendus Hebd Seances Acad Sci 248 (11): 1721–22. Available at http://gallica.bnf.fr/ark:/12148/bpt6k32002/f1759.chemindefer, accessed at Dec 11, 2011.

Leskinen S, Ryynänen M, Kirkinen P, Heinonen S, Saarikoski S. Sikiön ei-perinnöllisen kromosomivian toistumisriski. Lääketieteellinen Aikakauskirja Duodecim. 1996;112(13):1172 (In Finnish).

Leunen K, Hall DR, Odendaal HJ, Grové D. The profile and complications of women with placental abruption and intrauterine death. J Trop Pediatr. 2003 Aug;49(4):231-4.

Liao AW, Heath V, Kametas N, Spencer K, Nicolaides KH. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. Hum Reprod. 2001 Jul;16(7):1501-4.

Lim JH, Kim SY, Park SY, Yang JH, Kim MY, Ryu HM. Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors. Obstet Gynecol. 2008 Jun;111(6):1403-9.

Lin TM, Halbert SP, Spellacy WN. Measurement of pregnancy-associated plasma proteins during human gestation. J Clin Invest. 1974 Sep;54(3):576-82.

Liu SS, Lee FK, Lee JL, Tsai MS, Cheong ML, She BQ, Chen SC. Pregnancy outcomes in unselected singleton pregnant women with an increased risk of first-trimester Down's syndrome. Acta Obstet Gynecol Scand. 2004 Dec;83(12):1130-4.

Loudon MM, Day RE, Duke EM. Thyroid dysfunction in Down's syndrome. Arch Dis Child. 1985 Dec;60(12):1149-51.

Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, Berkowitz RL, Gross SJ, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Dukes K, Bianchi DW, Rudnicka AR, Hackshaw AK, Lambert-Messerlian G, Wald NJ, D'Alton ME. First- and Second-Trimester Evaluation of Risk (FASTER) Research Consortium. First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med. 2005 Nov 10;353(19):2001-11.

Mandal D, Manda S, Rakshi A, Dey RP, Biswas SC, Banerjee A. Maternal obesity and pregnancy outcome: a prospective analysis. J Assoc Physicians India. 2011 Aug;59:486-9.

Marlow N, Wolke D, Bracewell MA, Samara M; EPICure Study Group. Neurologic and developmental disability at six years of age after extremely preterm birth. N Engl J Med. 2005 Jan 6;352(1):9-19.

Matias A, Gomes C, Flack N, Montenegro N, Nicolaides KH. Screening for chromosomal abnormalities at 10-14 weeks: the role of ductus venosus blood flow. Ultrasound Obstet Gynecol. 1998 Dec;12(6):380-4.

Mavalankar DV, Gray RH, Trivedi CR. Risk factors for preterm and term low birthweight in Ahmedabad, India. Int J Epidemiol. 1992 Apr;21(2):263-72.

Maynard S, Epstein FH, Karumanchi SA. Preeclampsia and angiogenic imbalance. Annu Rev Med. 2008;59:61-78.

Melamed N, Aviram A, Silver M, Peled Y, Wiznitzer A, Glezerman M, Yogev Y. Pregnancy course and outcome following blunt trauma. J Matern Fetal Neonatal Med. 2011 Dec 22. [Epub ahead of print]

Mendoza-Caamal EC, Grether-González P, Hernández-Gómez M, Guzmán-Huerta M, Aguinaga-Ríos M. [Birth defects associated with increased nuchal translucency]. [Article in Spanish] Ginecol Obstet Mex. 2010 Oct;78(10):533-9.

Merkatz I, Nitowsky H, Macri J, Johnson W. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol 1984;148:886-94.

Mikkelsen M. Epidemiology of trisomy 21: population, peri- and antenatal data. Hum Genet Suppl. 1981;2:211-26.

Mishell DR Jr, Davajan V. Quantitative immunologic assay of human chorionic gonadotropin in normal and abnormal pregnancies. Am J Obstet Gynecol. 1966 Sep 15;96(2):231-9.

Montagnana M, Franchi M, Danese E, Gotsch F, Guidi GC. Disseminated intravascular coagulation in obstetric and gynecologic disorders. Semin Thromb Hemost. 2010 Jun;36(4):404-18. Epub 2010 Jul 7.

Murphy A, Platt LD. First-trimester diagnosis of body stalk anomaly using 2- and 3-dimensional sonography. J Ultrasound Med. 2011 Dec;30(12):1739-43.

Nakhai-Pour HR, Rey E, Bérard A. Antihypertensive medication use during pregnancy and the risk of major congenital malformations or small-for-gestational-age newborns. Birth Defects Res B Dev Reprod Toxicol. 2010 Apr;89(2):147-54.

National Supervisory Authority for Health and Welfare. Termination of pregnancy. 2012 (In Finnish). Available at: http://www.valvira.fi/luvat/raskauden_ keskeyttaminen. Accessed Feb 27, 2012.

Nicolaides K, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. Br Med J 1992;304:867-9.

Nicolaides KH, Heath V, Cicero S. Increased fetal nuchal translucency at 11-14 weeks. Prenat Diagn. 2002 Apr;22(4):308-15.

Nicolaides KH. The NT Book. London, 2004. The Fetal Medicine Foundation. Available at: http://www.fetalmedicine.com/fmf/FMF-English.pdf. Accessed Feb 12, 2012.

Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. Ultrasound Obstet Gynecol. 2005 Mar;25(3):221-6.

Nicolaides KH. Turning the pyramid of prenatal care. Fetal Diagn Ther. 2011;29(3):183-96. doi: 10.1159/000324320. Epub 2011 Mar 8.

Nicolaidis P, Petersen MB. Origin and mechanisms of non-disjunction in human autosomal trisomies. Hum Reprod. 1998 Feb;13(2):313-9.

Niemimaa M, Suonpää M, Perheentupa A, Seppälä M, Heinonen S, Laitinen P, Ruokonen A, Ryynänen M. Evaluation of first trimester maternal serum and ultrasound screening for Down's syndrome in Eastern and Northern Finland. Eur J Hum Genet. 2001 Jun;9(6):404-8.

Norgaard-Pedersen B, Bagger P, Bang J, Fischer-Rasmunssen W, Gad C, Hasch E, ym. Ma- ternal-serum-alphafetoprotein screening for fetal malformations in 28 062 pregnancies. A four-year experience from a low-risk area. Acta Obstet Gynecol Scand 1985;64(6):511-4.

Orlandi F, Damiani G, Hallahan TW, Krantz DA, Macri JN. First-trimester screening for fetal aneuploidy: biochemistry and nuchal translucency. Ultrasound Obstet Gynecol. 1997 Dec;10(6):381-6.

Orlandi F, Rossi C, Allegra A, Krantz D, Hallahan T, Orlandi E, Macri J. First trimester screening with free beta-hCG, PAPP-A and nuchal translucency in pregnancies conceived with assisted reproduction. Prenat Diagn. 2002 Aug;22(8):718-21.

Ostesen M. Optimisation of antirheumatic drug treatment in pregnancy. Clin Pharmacokinet. 1994 Dec;27(6):486-503.

Oyelese Y, Ananth CV. Placental abruption. Obstet Gynecol. 2006 Oct;108(4):1005-16.

Ozkaya O, Sezik M, Ozbasar D, Kaya H. Abnormal ductus venosus flow and tricuspid regurgitation at 11-14 weeks' gestation have high positive predictive values for increased risk in first-trimester combined screening test: results of a pilot study. Taiwan J Obstet Gynecol. 2010 Jun;49(2):145-50.

Pajkrt E, van Lith JM, Mol BW, Bleker OP, Bilardo CM. Screening for Down's syndrome by fetal nuchal translucency measurement in a general obstetric population. Ultrasound Obstet Gynecol. 1998 Sep;12(3):163-9.

Palomaki GE, Lee JE, Canick JA, McDowell GA, Donnenfeld AE; ACMG Laboratory Quality Assurance Committee. Technical standards and guidelines: prenatal screening for Down syndrome that includes first-trimester biochemistry and/or ultrasound measurements. Genet Med. 2009;11:669-81.

Pandya PP, Brizot ML, Kuhn P, Snijders RJ, Nicolaides KH. First-trimester fetal nuchal translucency thickness and risk for trisomies. Obstet Gynecol. 1994 Sep;84(3):420-3.

Papageorgiou EA, Karagrigoriou A, Tsaliki E, Velissariou V, Carter NP, Patsalis PC. Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21. Nat Med. 2011 Apr;17(4):510-3. Epub 2011 Mar 6.

Pariente G, Wiznitzer A, Sergienko R, Mazor M, Holcberg G, Sheiner E. Placental abruption: critical analysis of risk factors and perinatal outcomes. J Matern Fetal Neonatal Med. 2011 May;24(5):698-702. Epub 2010 Sep 9.

Pell JP, Smith GC, Walsh D. Pregnancy complications and subsequent maternal cerebrovascular events: a retrospective cohort study of 119,668 births. Am J Epidemiol. 2004 Feb 15;159(4):336-42.

PerkinElmer. Autodelfia. Instructions for use. PAPP-A B098-201. PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.

PerkinElmer. Autodelfia. Instructions for use. Free hCGβ B097-101. PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland.

Petersen MB, Mikkelsen M. Nondisjunction in trisomy 21: origin and mechanisms. Cytogenet Cell Genet. 2000;91(1-4):199-203.

Poon LC, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. Hypertension. 2009 May;53(5):812-8. Epub 2009 Mar 9.

Prats P, Rodríguez I, Nicolau J, Comas C. Early first-trimester free-β-hCG and PAPP-A serum distributions in monochorionic and dichorionic twins. Prenat Diagn. 2012 Jan;32(1):64-9. doi: 10.1002/pd.2902.

Ritvanen, Sirkiä. Congenital anomalies 1993–2008, Statistical report. National Institute for Health and Welfare 2011. ISSN 1798-0887. 7/2011. Available at: http://www.stakes.fi/
tilastot/tilastotiedotteet/2011/Tr07_11.pdf. Accessed Feb 28, 2012.

Roberts WE, Morrison JC, Hamer C, Wiser WL. The incidence of preterm labor and specific risk factors. Obstet Gynecol. 1990 Jul;76(1 Suppl):85S-89S.

Rodis JF, Egan JF, Craffey A, Ciarleglio L, Greenstein RM, Scorza WE. Calculated risk of chromosomal abnormalities in twin gestations. Obstet Gynecol. 1990 Dec;76(6):1037-41.

Roizen NJ, Wolters C, Nicol T ym. Hearing loss in children with Down syndrome. J Pediatr 1993;123:S9-12.

Rossi AC, Mullin PM. Prevention of pre-eclampsia with low-dose aspirin or vitamins C and E in women at high or low risk: a systematic review with meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2011 Sep;158(1):9-16.

Ryynänen M, Tekay A, Niemimaa M, Taipale P, Heinonen S. Miten Down-seulontaa parannetaan (In Finnish). Lääketieteellinen Aikakauskirja Duodecim 2004;120(3):354-5.

Saftlas AF, Olson DR, Atrash HK, Rochat R, Rowley D. National trends in the incidence of abruptio placentae, 1979-1987. Obstet Gynecol. 1991 Dec;78(6):1081-6.

Saling E, Schreiber M, al-Taie T. A simple, efficient and inexpensive program for preventing prematurity. J Perinat Med. 2001;29(3):199-211.

Schuchter K, Hafner E, Stangl G, Metzenbauer M, Höfinger D, Philipp K. The first trimester "combined test" for the detection of Down syndrome pregnancies in 4939 unselected pregnancies. Prenat Diagn 2002;22:211-5.

Sebire NJ, Bianco D, Snijders RJ, Zuckerman M, Nicolaides KH. Increased fetal nuchal translucency thickness at 10-14 weeks: is screening for maternal-fetal infection necessary? Br J Obstet Gynaecol. 1997 Feb;104(2):212-5.

Seppala M, Ruoslahti E. Alpha-fetoprotein in antenatal diagnosis. Lancet. 1973 Jan 20;1(7795):155.

Sibai BM, Ramadan MK, Usta I, Salama M, Mercer BM, Friedman SA. Maternal morbidity and mortality in 442 pregnancies with hemolysis, elevated liver enzymes, and low platelets (hellp syndrome) *Am J Obstet Gynecol.* 1993;169:1000–1006.

Smith GC, Pell JP, Walsh D. Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. Lancet. 2001 Jun 23;357(9273):2002-6.

Smith GC, Stenhouse EJ, Crossley JA, Aitken DA, Cameron AD, Connor JM. Early pregnancy levels of pregnancy-associated plasma protein a and the risk of intrauterine growth restriction, premature birth, preeclampsia, and stillbirth. J Clin Endocrinol Metab. 2002 Apr;87(4):1762-7.

Smith HO, Kohorn E, Cole LA. Choriocarcinoma and gestational trophoblastic disease. Obstet Gynecol Clin North Am. 2005 Dec;32(4):661-84.

Snijders RJ, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age- and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol. 1999 Mar;13(3):167-70.

Souka AP, Snijders RJ, Novakov A, Soares W, Nicolaides KH. Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10-14 weeks of gestation. Utrasound Obstet Gynecol 1998;11:388-400.

South Ostrobothnia Central Hospital District (Etelä-Pohjanmaan sairaanhoitopiiri). Palveluhinnasto 2012. Available at: http://www.epshp.fi/files/55/ Palveluhinnasto_2012.pdf. Accessed Feb 12, 2012.

South Savonia Central Hospital District (Etelä-Savon sairaanhoitopiirin kuntayhtymä). Palveluhinnasto 2011. Available at: http://www.esshp.fi/ downloader.asp?id=4852&type=1. Accessed Feb 12, 2012.

Spencer K, Coombes EJ, Mallard AS, Ward AM. Free beta human choriogonadotropin in Down's syndrome screening: a multicentre study of its role compared with other biochemical markers. Ann Clin Biochem. 1992 Sep;29 (Pt 5):506-18.

Spencer K. Second trimester prenatal screening for Down's syndrome using alpha-fetoprotein and free beta hCG: a seven year review. Br J Obstet Gynaecol. 1999 Dec;106(12):1287-93.

Spencer K. Screening for trisomy 21 in twin pregnancies in the first trimester using free beta-hCG and PAPP-A, combined with fetal nuchal translucency thickness. Prenat Diagn. 2000 Feb;20(2):91-5.

Spencer K, Nicolaides KH. A first trimester trisomy 13/trisomy 18 risk algorithm combining fetal nuchal translucency thickness, maternal serum free beeta-hCG and PAPP-A. Prenat Diagn 2002;22:877-9.

Spencer K, Spencer C, Power M, Dawson C, Nicolaides K. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years prospective experience. Br J Obstet Gynaecol 2003;110:281-6.

Spencer K, Nicolaides K. Screening for trisomy 21 in twins using first trimester ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years experience. Br J Obstet Gynaecol 2003;110:276-80.

Spencer K, Cicero S, Atzei A, Otigbah C, Nicolaides KH. The influence of maternal insulin-dependent diabetes on fetal nuchal translucency thickness and first-trimester maternal serum biochemical markers of aneuploidy. Prenat Diagn. 2005 Oct;25(10):927-9.

Spencer K, Cowans NJ, Avgidou K, Molina F, Nicolaides KH. First-trimester biochemical markers of aneuploidy and the prediction of small-for-gestational age fetuses. Ultrasound Obstet Gynecol. 2008 Jan;31(1):15-9. (a)

Spencer K, Cowans NJ, Molina F, Kagan KO, Nicolaides KH. First-trimester ultrasound and biochemical markers of aneuploidy and the prediction of preterm or early preterm delivery. Ultrasound Obstet Gynecol. 2008 Feb;31(2):147-52. (b)

Steele M, Breg W. Chromosome analysis of human amniotic-fluid cells. Lancet 1966;1:383-5.

Steele GL, Currie WD, Yuen BH, Jia XC, Perlas E, Leung PC. Acute stimulation of human chorionic gonadotropin secretion by recombinant human activin-A in first trimester human trophoblast. Endocrinology. 1993 Jul;133(1):297-303.

Steinborn A, Seidl C, Sayehli C, Sohn C, Seifried E, Kaufmann M, Schmitt E. Anti-fetal immune response mechanisms may be involved in the pathogenesis of placental abruption. Clin Immunol. 2004 Jan;110(1):45-54.

Stenman UH, Alfthan H, Myllynen L, Seppälä M. Ultrarapid and highly sensitive time-resolved fluoroimmunometric assay for chorionic gonadotropin. Lancet. 1983 Sep 17;2(8351):647-9.

Sun IY, Overgaard MT, Oxvig C, Giudice LC. Pregnancy-associated plasma protein A proteolytic activity is associated with the human placental trophoblast cell membrane. J Clin Endocrinol Metab. 2002 Nov;87(11):5235-40.

Tabor A, Alfirevic Z. Update on procedure-related risks for prenatal diagnosis techniques. Fetal Diagn Ther. 2010;27(1):1-7. Epub 2009 Dec 24.

Taipale P, Hiilesmaa V, Salonen R, Ylöstalo P. Increased nuchal translucency as a marker for fetal chromosomal defects. N Engl J Med. 1997 Dec 4;337(23):1654-8.

Taipale P, Hiilesmaa V. Predicting delivery date by ultrasound and last menstrual period in early gestation. Obstet Gynecol 2001;97:189-94.

Taipale P, Ammälä M, Salonen R, Hiilesmaa V. Learning curve in ultrasonographic screening for selected fetal structural anomalies in early pregnancy. Obstet Gynecol. 2003 Feb;101(2):273-8.

Tekay A, THL. Raskausajan ultraääniseulontojen laatuvaatimukset (In Finnish). Available at: http://www.thl.fi/thl-client/pdfs/888e4255-2762-4111-a0c3-4bfbf965c26c. Accessed Feb 27, 2012.

Teperi J. Multi method approach to the assessment of data quality in the Finnish Medical Birth Registry. J Epidemiol Community Health. 1993 Jun;47(3):242-7.

Terho, A. Trisomiaseulonnan laatukriteerit ja alueelliset erot Suomessa (In Finnish). Oulun Yliopisto, Oulu 2002, 11-13.

Tikkanen M. Placental abruption: epidemiology, risk factors and consequences. Acta Obstet Gynecol Scand. 2011 Feb;90(2):140-9. doi: 10.1111/j.1600-0412.2010.01030.x. Epub 2010 Dec 7.

Toivonen S, Heinonen S, Anttila M, Kosma V-M, Saarikoski S. Reproductive risk factors, Doppler findings and outcome of affected births in placental abruption – a population-based analysis. Am J Perinatology 2002;19:451-59.

Toivonen S, Keski-Nisula L, Saarikoski S, Heinonen S. Risk of placental abruption in first-degree relatives of index patients. Clin Genet. 2004 Sep;66(3):244-6.

Trivedi NA. A meta-analysis of low-dose aspirin for prevention of preeclampsia. J Postgrad Med. 2011 Apr-Jun;57(2):91-5.

Tul N, Novak-Antolic Z. Serum PAPP-A levels at 10-14 weeks of gestation are altered in women after assisted conception. Prenat Diagn. 2006 Dec;26(13):1206-11.

Tul N, Pusenjak S, Osredkar J, Spencer K, Novak-Antolic Z. Predicting complications of pregnancy with first-trimester maternal serum free-betahCG, PAPP-A and inhibin A. Prenat Diagn. 2003 Dec 15;23(12):990-6.

Vaisbuch E, Whitty JE, Hassan SS, Romero R, Kusanovic JP, Cotton DB, Sorokin Y, Karumanchi SA. Circulating angiogenic and antiangiogenic factors in women with eclampsia. Am J Obstet Gynecol. 2011 Feb;204(2):152.e1-9. Epub 2010 Nov 9.

Valinen Y, Laitinen P, Ranta J, Ignatius J, Jarvela I, Ryynänen M. Effect of a new marker, ADAM12, on Down risk figures in first trimester screening. J Matern Fetal Neonatal Med. 2009 Jul;22(7):602-7.

van Echten-Arends J, Mastenbroek S, Sikkema-Raddatz B, Korevaar JC, Heineman MJ, van der Veen F, Repping S. Chromosomal mosaicism in human preimplantation embryos: a systematic review. Hum Reprod Update. 2011 Sep-Oct;17(5):620-7. Epub 2011 Apr 29.

van Faassen E, Niemelä O. The biochemistry of prenatal alcohol exposure. In: Pregnancy and Alcohol Consumption. Hoffman Joshua D. Nova Science Publishers, Inc., New York 2011, pp 1–65. ISBN: 978-1-61761-122-3.

Viisainen. Stakesin perhesuunnittelun ja äitiyshuollon asiantuntijaryhmä. Seulontatutkimukset ja yhteistyö äitiyshuollossa (In Finnish). ISBN 951-33-0922-3. Available at: http://www.stakes.fi/verkkojulkaisut/Muut/op34_1999.pdf. Accessed Jan 19, 2012.

Vuori, Gissler. Perinatal statistics: parturients, deliveries and Newborns 2010. National Institute for Health and Welfare 2011. ISSN 1798-0887. 27/2011. Available at: http://www.stakes.fi/tilastot/tilastotiedotteet/2010/Tr06_10.pdf. Accessed Feb 28, 2012.

Wald NJ, Cuckle HS, Brock JH, Peto R, Polani PE, Woodfroed FP. Maternal serum-alpha- fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of the U.K. collaborative study on alpha-fetoprotein in relation to neural- tube defects. Lancet 1977;1(8026):1323-32.

Wald NJ, Cuckle HS, Densem JW, Nanchahal K, Royston P, Chard T, Haddow JE, Knight GJ, Palomaki GE, Canick JA. Maternal serum screening for Down's syndrome in early pregnancy. BMJ. 1988 Oct 8;297(6653):883-7.

Wald NJ, Densem JW, Cheng R, Collishaw S. Maternal serum free alpha- and free beta- human chorionic gonadotropin in pregnancies with insulin-depedendent diabetes mellitus: implications for screening for Down's syndrome. Prenat Diagn 1994;14(9):835-7.

Wald NJ, George L, Smith D, Densem JW, Petterson K. Serum screening for Down's syndrome between 8 and 14 weeks of pregnancy. International Prenatal Screening Research Group. Br J Obstet Gynaecol. 1996 May;103(5):407-12. (a)

Wald NJ, Densem JW, George L, Muttukrishna S, Knight PG. Prenatal screening for Down's syndrome using inhibin-A as a serum marker. Prenat Diagn. 1996 Feb;16(2):143-53. (b)

Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson A. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). Health Technol Assess 2003;7(11):1-88.

Wang YN, Yang Z. [Multivariate analysis of risk factors with placental abruption in preeclampsia]. [Article in Chinese] Zhonghua Fu Chan Ke Za Zhi. 2010 Nov;45(11):825-8.

Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkins K, Platt L, Mahoney M, Johnson A, Hogge WA, Wilson RD, Mohide P, Hershey D, Krantz D, Zachary J, Snijders R, Greene N, Sabbagha R, MacGregor S, Hill L, Gagnon A, Hallahan T, Jackson L; First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening (BUN) Study Group. First-trimester screening for trisomies 21 and 18. N Engl J Med. 2003 Oct 9;349(15):1405-13.

Weinstein L. Syndrome of hemolysis, elevated liver enzymes, and low platelet count: A severe consequence of hypertension in pregnancy. Am J Obstet Gynecol. 1982;142:159–167.

Whitlow BJ, Chatzipapas IK, Lazanakis ML, Kadir RA, Economides DL. The value of sonography in early pregnancy for the detection of fetal abnormalities in an unselected population. Br J Obstet Gynaecol 1999;106:929-36.

WHO recommendations for Prevention and treatment of pre-eclampsia and eclampsia. Summary of Recommendations. Availabe at: http://whqlibdoc.who.int/hq/2011WHO_RHR_11.30_eng.pdf. Accessed Feb 6, 2012.

Wilkins-Haug L, Roberts DJ, Morton CC. Confined placental mosaicism and intrauterine growth retardation: a case-control analysis of placentas at delivery. Am J Obstet Gynecol. 1995 Jan;172(1 Pt 1):44-50.

Wilson JMG, Jungner G. Principles and practice of screening for disease. Geneva: WHO; 1968. Available at: http://whqlibdoc.who.int/php/WHO_PHP_34.pdf. Accessed Feb 28, 2012.

Woolcock J, Hennessy A, Xu B, Thornton C, Tooher J, Makris A, Ogle R. Soluble Flt-1 as a diagnostic marker of pre-eclampsia. Aust N Z J Obstet Gynaecol. 2008 Feb;48(1):64-70.

Working group appointed by the Finnish Medical Society Duodecim and The Finnish Association of Doctors in Developmental Disability. Current Care Summary. Down's syndrome. 29.12.2010. Available at: http://www.kaypahoito.fi/web/kh/suositukset/naytaartikkeli/tunnus/ccs00007, accessed Dec 11, 2011.

Wright D, Spencer K, Kagan K K, Tørring N, Petersen OB, Christou A, Kallikas J, Nicolaides KH. First-trimester combined screening for trisomy 21 at 7-14 weeks' gestation. Ultrasound Obstet Gynecol. 2010 Oct;36(4):404-11.

Yanney M, Marlow N. Paediatric consequences of fetal growth restriction. Semin Fetal Neonatal Med. 2004 Oct;9(5):411-8.

Jenni Ranta-Ylikulju

First Trimester Down´s Syndrome Screening

The Role of Biochemistry

Maternal and foetal characteristics affect the levels of the biochemical markers in first trimester Down's syndrome screening. Pregnancy associated plasma protein A is not only the best marker to screen for Down's syndrome, but also a significant marker for subfertility and adverse pregnancy outcomes, such as preterm delivery, preeclampsia and small for gestational age infants. In this thesis, the pregnancy associated plasma protein A was examined to elucidate how its concentration behaves in relation to maternal and gestational ages, as well as how these factors affect the detection rate of Down's syndrome.

UNIVERSITY OF EASTERN FINLAND

Publications of the University of Eastern Finland
Dissertations in Health Sciences

ISBN 978-952-61-0769-1