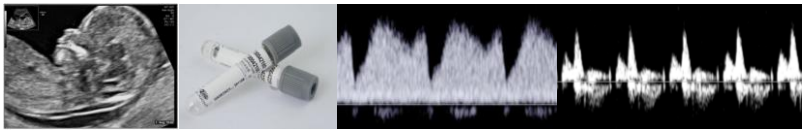
Faculty of Health Sciences
University of Copenhagen

Screening for trisomy 21 in Denmark;

Evaluation of the current and possible future strategies



PhD thesis

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The PhD defence will take place Friday the 13th of April 2012, Auditorium B, Teilum, Rigshospitalet, Copenhagen.

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Finally, I want to thank my family and friends for their interest and support.
And to Kim, Clara and Sofie: I love you so much.

Charlotte Kvist Ekelund
Copenhagen , March 2012

Content

Preface and aims

The present Ph.D. thesis is based on work carried out during my employment as a research fellow at the Department of Fetal Medicine, Rigshospitalet, Copenhagen University Hospital, Denmark during the period 2007-2011. The work was primarily supervised by professor, DMsc Ann Tabor, Department of Fetal Medicine, Rigshospitalet, Copenhagen. Head of Fetal Medicine Unit, Ph.D. Olav Bjørn Petersen and Head of Department of Clinical Genetics, DMSc Ida Vogel, Aarhus University Hospital have in addition contributed to the scientific plan and collection of data.

The Ph.D. thesis is based on the following original papers

I. **Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study.** Ekelund CK, Jorgensen FS, Petersen OB, Sundberg K, Tabor A, BMJ 2008; 337:a2547

II. **First trimester screening for Trisomy 21 in Denmark: Implications on detection and birth rates of Trisomy 18 and Trisomy 13**. Ekelund CK, Petersen OB, Skibsted L, Kjaergaard S, Vogel I, Tabor A. Ultrasound Obstet Gynecol 2011;38(2):140-4

III. **A prospective study evaluating the performance of first trimester combined screening for trisomy 21 using repeated sampling of the maternal serum markers PAPP-A and free β-hCG.** Ekelund CK, Wright D, Ball S, Kirkegaard I, Nørgaard P, Sørensen S, Friis-Hansen L, Jørgensen FS, Tørring N, Bech BH, Petersen OB, Tabor A. Submitted march 2012 to Ultrasound in Obstetrics and Gynecology.

IV. **Screening performance for trisomy 21 comparing first trimester combined screening and a first trimester contingent screening protocol including ductus venosus and tricuspid flow**. Ekelund CK, Petersen OB, Sundberg K, Pedersen F, Vogel I, Tabor A. Submitted October 2011 to Prenatal Diagnosis. Currently undergoing revision.

The aims of the Ph.D. thesis were:

1. To evaluate the first trimester combined screening programme for trisomy 21 in Denmark implemented following the new national guideline in 2004
2. To assess whether the national screening programme for trisomy 21 has changed the gestational age at which trisomy 18 and trisomy 13 are detected
3. To investigate if access to a double set of the maternal serum markers PAPP-A and free β-hCG can improve screening performance
4. To compare routine first trimester screening for trisomy 21 with a contingent screening protocol including two new ultrasound markers; abnormal ductus venosus flow and tricuspid regurgitation.

List of abbreviations

AC: Amniocentesis

ADAM 12: A Disintegrin And Metalloprotease 12

AFP: Alpha Foeto Protein

CRL: Crown Rump Length

CVS: Chorionic villus sample

DR: Detection rate

FPR: False positive rate

FMF: Fetal Medicine Foundation

Free β-hCG: Free beta human Chorion Gonadotrophin

IGF: Insulin-like-growth-factor

IGFBP-4: Insulin-like-growth-factor-binding-protein-4

LMP: Last Menstrual Period

LR: Likelihood Ratio

MoM: Multiple of the Median

NT: Nuchal Translucency

OSCAR: One Stop Clinic for Assessment of Risk

PAPP-A: Pregnancy-Associated-Plasma-Protein-A

SP1: Schwangerschafts Protein 1

Introduction

In 2004 the Danish National Board of Health issued a new guideline on prenatal screening[1]. In contrast to the previous guideline from 1994 the new guideline recommended that all pregnant women should be offered information about prenatal examinations in pregnancy. The optional prenatal examinations were a screening test for trisomy 21 (Down syndrome) in the first trimester of pregnancy (the combined test; a nuchal translucency scan and a blood test) and a scan in the second trimester (malformation scan). The new guideline was well accepted by local politicians, hospitals administrators, doctors and the pregnant women. The new guideline was implemented gradually over the next 1½ year and by June 2006 the screening programme was considered an offer to all Danish pregnant women[2].

This Ph.D. thesis focuses on the implemented first trimester screening programme for trisomy 21. Our aim was to follow up on the impact of the new screening strategy in terms of performance of the programme in a national cohort. In addition we wished to explore if additional ultrasound and biochemical markers could potentially improve the screening performance of the programme.

The background section of the Ph.D. thesis presents the overall principles of prenatal screening. Emphasis is placed on the first trimester combined screening which is now the offer to pregnant women in Denmark. Review of relevant literature on ultrasound and biochemical markers used in screening for trisomy 21 is presented. The unique tools available for performing epidemiological research in Denmark are discussed in the section on epidemiological research in fetal medicine and are followed by presentation and discussion of results from the four papers, on which the thesis is based.

Background

Prenatal diagnosis and screening for chromosomal abnormalities

Prenatal diagnostic tests

Chromosomal abnormalities are the leading cause of developmental delay in children and trisomy 21, trisomy 18, trisomy 13 and sex chromosome aberrations are the most commonly occurring chromosomal abnormalities[3;4]. Researchers have during the last 30 years worked intensively to develop a non-invasive prenatal diagnostic test for chromosomal abnormalities. Progress has been made by using techniques which examine fetal cells or cell-free fetal DNA/RNA in maternal plasma[5]. It may become possible in the coming years to diagnose a fetus with trisomy 21 by examining the maternal blood, but currently, the only available diagnostic tests we can offer the pregnant women are one of two invasive diagnostic tests; amniocentesis (AC) or chorionic villus sample (CVS). The tests are based on direct collection of fetal or placental cells followed by a diagnostic chromosome analysis.

Amniocentesis was the first diagnostic invasive test to be introduced more than 40 years ago[6]. It was traditionally performed blindly around gestational week 16-17, but during the 1970'ies and 80'ies it became more and more common to perform the test under ultrasound guidance as first described by Bang et Nordtheved[7]. The procedure related risk of pregnancy loss associated with AC is 1%[8]. Attempts have been made to offer AC at earlier gestational ages, but an increased risk of fetal loss and higher incidence of club feet have been reported[9]. The recommendations therefore remain not to perform the procedure until week 15+3[10].

Chorionic villus sampling was developed in the 1980'ies as an alternative to AC and has become a widely used method for prenatal diagnosis in the first trimester[11]. It can be done from week 10+0 either transabdominally or transcervically. Before gestational week 10 there may be an increased risk of limb reduction defects[12]. Transabdominal CVS is reported to have a pregnancy loss rate comparable to AC performed in the second trimester, while transcervical CVS is likely to be associated with a significantly higher risk of miscarriage[13-15]. The transabdominally performed CVS has therefore become first choice in the majority of fetal medicine centres performing prenatal invasive tests, as access to early diagnosis is preferred by most women[16]. In addition to the information on 1% miscarriage risk pre-test counselling should also include information about a 1% risk of receiving an inconclusive result from a CVS[17]. The placenta can in some cases contain an abnormal cell line not present in the fetus (named placental mosaicism). In any cases with a CVS

mosaicism result it is thus recommended to perform an AC in order to determine if the mosaicism is confined to the placenta or also present in the fetus[10].

Traditionally collection of fetal or placental cells by AC or CVS has been followed by ***conventional karyotyping*** of long term cultured cells. This test provides the couple with the fetal karyotype in approximately 2 weeks[4]. Fortunately during the last decade rapid targeted aneuploidy testing has been widely implemented in routine prenatal diagnosis. Methods like interphase fluorescence in-situ hybridization (iFISH), quantitative fluorescence polymerase chain reaction (QF-PCR) and multiplex ligation-dependent probe amplification (MLPA) can within 24-48 hours detect aneuploidy of chromosome 13, 18, 21 and X and Y in prenatal samples. They have become a reliable supplement to the conventional karyotyping[18;19].

Prenatal screening tests

Invasive tests are costly and they pose as described above an inherent risk of procedure-related complications including fetal loss. It thus seems reasonable to reserve prenatal diagnostic tests to pregnancies with a high risk of chromosomal abnormalities. Prenatal screening tests have consequently been developed to identify women with high risk pregnancies, for whom the offer of invasive testing would be appropriate.

Screening has been defined by Wald and Cuckle as "the systematic application of a test, to identify subjects at sufficient risk of a specific disorder to benefit from further investigation or direct preventive action, among persons who have not sought medical attention on account of symptoms of that disorder" [20].

To assess the ***performance*** and to compare different screening tests it is essential to know how well the tests discriminate affected from unaffected individuals. For this purpose the ***detection rate*** (proportion of affected individuals yielding a positive result) and ***false positive rate*** (proportion of unaffected individuals yielding a positive result) have to be calculated or estimated. The ***screen positive rate*** is also often used when assessing the performance of screening for chromosomal abnormalities, as this is an easy parameter to monitor without having access to the final outcome of the pregnancy. In prenatal screening where the prevalence of any chromosomal abnormality is low, the false positive rate and screen positive rate are often quite similar. In screening for chromosomal abnormalities the screen positive rate directly reflects the proportion of pregnant women who will be offered a diagnostic invasive test. Thus the screen positive rate is, in cases

where screen positive women prefer to continue with an invasive test, an indirect measurement of the ***invasive testing rate***.

It is also useful to calculate or estimate "the odds of being affected given a positive result" (OAPR) for the screening test, which is the ratio of the number of true positives to the number of false positives.

Unlike the detection rate and false positive rate the OAPR is dependent on the prevalence of the disorder being screened for, and is thus not a property related only to the test itself but a parameter dependent on the population in which the test is applied[20]. Figure 1 shows the terms and calculations used in describing performance of screening tests.

	Affected	**Unaffected**	
Test positive	a	b	a+b
Test negative	c	d	c+d
	a+c	b+d	a+b+c+d

Detection rate (sensitivity)	a/a+c
Screen positive rate	a+b/a+b+c+d
False positive rate (1-specificity)	b/b+d
Odds of being affected given a screen positive result	a:b

Figure 1: Definition of screening performance parameters[20;21]

The OAPR can also be calculated using the so called likelihood ratio (LR). The LR is given as the detection rate divided by the false positive rate, and the OAPR is expressed as the prevalence of the disease multiplied by the LR[20].

In screening tests a ***cut off*** is often used to divide the screened population into "screen positive" and "screen negative". If high levels of a marker may be related to disease individuals with a test value above a certain level has increased risk of disease and are defined as screen positive. Individuals with a test level below the cut off will be referred to as screen negative. The detection rate, false positive rate and screen positive rate of the test are directly dependent on the chosen cut off. By looking at frequency distribution curves for affected and unaffected individuals for the

specific disorder the effect on screening performance when changing the cut off can be viewed as shown in figure 2A and 2B[20].

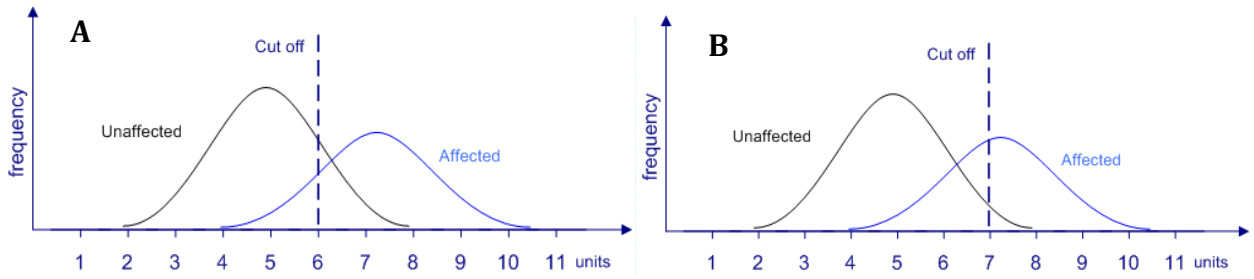

Figure 2: Gaussian relative frequency distribution curves for an imaginary screening marker in unaffected and affected fetuses. A and B show how detection and false positive rate are dependent on cut off. If screen positive is defined as having a value of 6 or above (figure 2A) ¾ of affected fetuses are detected, but a relatively large proportion of unaffected will be screened positive (high detection rate and high false positive rate). If the cut off is defined as 7 (figure 2B), the false positive rate will decrease at the expense of a decrease in detection rate.

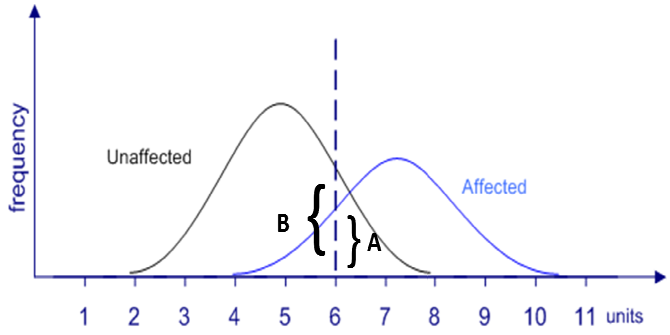

Figure 3: Gaussian relative frequency distribution curve. The likelihood ratio for a value of 6 is given by dividing the height of distribution curve for affected at this value (A) by the height of the unaffected curve (B). LR (value 6) = A/B

In many screening tests the screening variable is continuous, and then the calculated OAPR is not individualised, as it refers to the risk of being affected given the person has any value above the defined cut off. Using the frequency distribution curves it is possible to calculate individual LR for each possible test value and thereby provide an individual odds of being affected given that specific test result (figure 3). The specific LRs can in combination with a defined pre-test risk provide an exact post-test risk by using Bayes theorem. The principle is outlined in figure 4. If two screening variables/tests are not correlated the LRs can be multiplied to give a combined risk assessment based on more than a single test (figure 4).

In cases where the tests are correlated this can be taken into account thus requiring more advanced mathematics[22].

When screening for fetal chromosomal abnormalities the pre test risk is a known risk based on the maternal age and gestational age at time of screening (table 1). Screening variables can e.g. be proteins or hormones measured in maternal blood (biochemical markers) or anatomical variations measured by ultrasound (ultrasound markers), for which mean values of their respective normal distributions differ in unaffected and affected pregnancies. Thus the post test risk can e.g. be calculated as shown in figure 4[23;24].

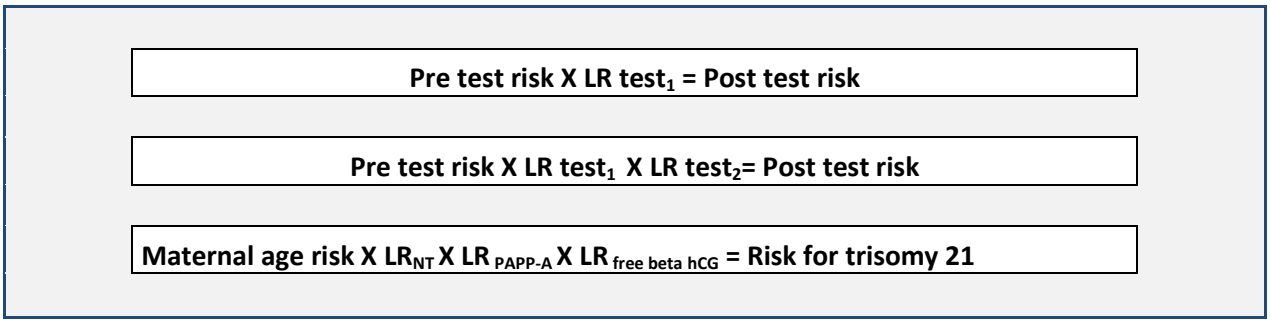

Figure 4: Bayes theorem for one or two screening variables/tests and when used in prenatal screening programmes[23;24].

Prenatal screening tests for trisomy 21

The risk of carrying a fetus with trisomy 21, 18 or 13 increases with increasing age of the mother. This association has been known since 1933[25]. In addition the risk is higher early in pregnancy compared to the risk around gestational week 40. This is due to a relatively high loss rate of fetuses with chromosomal abnormalities during pregnancy[26] (table 1).

	Estimated risk for trisomy 21			
	Gestational week			
Maternal age	**12**	**16**	**20**	**40**
20	1:1068	1:1200	1:1295	1:1527
25	1:946	1:1062	1:1147	1:1352
30	1:626	1:703	1:759	1:895
35	1:249	1:280	1:302	1:356
40	1:68	1:76	1:82	1:97

Table 1: Maternal age (in years) and gestational age related risk for trisomy 21, modified from "the 11-13+6 week scan" book, with permission from FMF.

As the invasive diagnostic tests were introduced in the 1970'ies screening for trisomy 21 using ***maternal age*** alone as a screening variable for chromosomal abnormalities began[27]. In Denmark this screening strategy offering pregnant women aged ≥ 35 years an invasive diagnostic test was recommended by the Danish National Board of Health up to 2004[28]. The screening performance using this strategy has been estimated to have a detection rate of 37%, a false positive rate of 10.6 % with an OAPR of 1:175 (calculated based on maternal age distribution 1998 in the Danish population), which is considered as a rather poor performance for a screening test [29]. As the false positive rate is almost the same as the screen positive rate, and if age was used as the only screening variable more than 10% of the pregnant women would be considered screen positive and therefore be offered an invasive diagnostic test. In Denmark where approx. 65,000 women are pregnant each year[30] it would be equivalent to 6-7000 invasive tests per year and consequently approximately 60-70 procedure related fetal losses. When screening for chromosomal abnormalities the screen positive rate should be as low as possible to keep the number of invasive tests at a minimum.

In the 1980'ies serum screening tests for trisomy 21 in the second trimester of pregnancy were developed and in 1988 Wald et al. described the ***triple test***, combining maternal age with three biochemical markers (Alfa Foeto Protein (AFP), estradiol and human Chorionic Gonadotrophin (hCG)) [31]. The screening performance for the triple test was much better than using maternal age alone and it was reported by Wald et al. to have a DR of 60% for a false positive rate of 5%[31]. In some parts of Denmark in the 1990'ies the triple test was offered routinely so to women aged < 35 years with results as least as good as predicted by models and shown in studies from centres in other countries[32;33].

In the beginning of the 1990'ies after the CVS was introduced as a diagnostic invasive procedure which could be performed in the first trimester of pregnancy, the ***first trimester screening tests*** followed accordingly. Pregnancy associated plasma protein A (PAPP-A) and free β human chorionic (free β-hCG) were described as first trimester markers for trisomy 21[34;35] and around the same time the nuchal translucency (fluid accumulated in the posterior region of fetal neck) in the first trimester and its relation to chromosomal abnormalities was described[36].

There was now a wide variety of screening tests available based on serum markers alone or on the combination of ultrasound and serum markers, and screening performance was reported to be significantly better than performance based on the maternal age criteria[27]. In Denmark there was a

strong professional wish for revision of the current national standards of prenatal screening and a working group under the Danish National Board of Health was established. In 2003 they issued a report including their recommendations[29]. As part of their work they compared the different screening strategies at hand at that time by using a standardised population of Danish pregnant women and available data from published work on the markers (statistical method as described by Wald et al. 1988[31])[29]. The screening performance for different screening strategies is shown in figure 5.

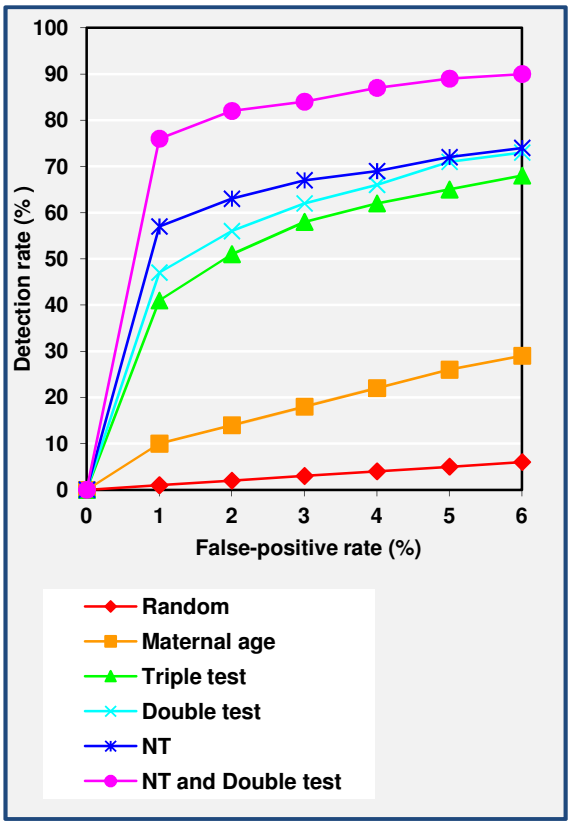

Figure 5: Receiver operating curves for different screening strategies for trisomy 21[29]

National guideline on prenatal screening

In 2004 the Danish National Board of Health issued a new guideline on prenatal screening and diagnostics[1]. The guideline was based on the report "prenatal diagnostics and risk assessments" from 2003 by the group referred to above[29].

Four major changes compared to the previous guideline were introduced.

- The guideline recommends that all pregnant women should have the offer of getting more information about prenatal screening and possible tests; ***"informed choice",*** instead of

sorting into high risk/low risk automatically by age without involving the pregnant woman herself.

- The guideline opens up for all pregnant women to have the choice of a risk assessment for trisomy 21 performed based on ultrasound scanning and measurement of two biochemical markers in the first trimester (***the first trimester combined screening test***).
- The importance of information about the possibilities of getting support through ***patient organisations*** in case a fetus is diagnosed with any abnormality is emphasized.
- The guideline focuses on follow up and measurement of the ***quality*** of the prenatal information, examinations and tests provided to the pregnant women.

The above listed changes were explained as a logic consequence of availability of new screening tests, which seemed superior to using the age criterion. In addition respect for the patients' autonomy and a need for lowering the number of prenatal invasive tests in Denmark were given as reasons for change in recommendations.

The guideline was well accepted by the pregnant women, the obstetricians and the politicians. The offer of first trimester combined risk assessment was gradually implemented all over the country. Training of sonographers and establishment of logistics around the collection of blood samples were initiated. In June 2006 all pregnant women in Denmark could choose to have a risk assessment for trisomy 21 performed using the first trimester combined screening strategy[2]. Denmark was one of the first countries in the world to reach a national consensus on prenatal screening including the offer of a free of charge first trimester combined screening test to all pregnant women.

In the following background section of the thesis a more detailed description of the first trimester combined screening test and its components is provided and possible new ways to improve the screening test are presented.

First trimester combined screening for trisomy 21

The first trimester combined screening test for trisomy 21 consists of one ultrasound marker; the nuchal translucency measurement and two independent biochemical markers measured in maternal blood; pregnancy associated plasma protein A (PAPP-A) and the free β-human Chorionic Gonadotrophin (free β-hCG).

Nuchal translucency

All fetuses have a small translucent area in the posterior region of the fetal neck which is easily visualised using ultrasound. It was originally observed when performing ultrasound examinations in the second or third trimester of pregnancy, named cystic hygroma or nuchal edema and was found to be related to malformations and chromosomal abnormalities[36-39].

In 1992 Nicolaides and co-workers introduced the term nuchal translucency (NT) as an accumulation of fluid behind the fetal neck in the first trimester (figure 6) and described the association between the thickness of the NT and abnormal fetal karyotype[40]. They reported that in a group of fetuses with an NT ≥3 mm 35% had a chromosomal abnormality in contrast to the group with an NT of less than 3 mm, where only 1% were chromosomally abnormal.

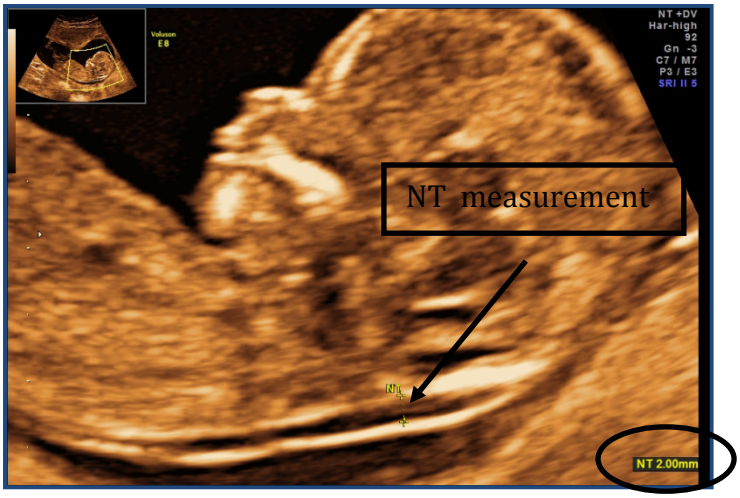

Figure 6. Mid sagittal profile of a fetus with measurement of the nuchal translucency thickness (2.0 mm)

In addition increased fetal NT has been demonstrated to be associated with cardiac malformations as first described by Hyett et al.[41], as well as many other fetal abnormalities and genetic syndromes[42-45]. In fetuses with increased NT the risk of an adverse outcome increases with increasing NT thickness. The risk of adverse outcome in fetuses with an NT ≥ 6.5mm is reported to be around 80-85%[44;45]. It should be emphasized though that also a normal fetus can have a thick

nuchal translucency in the first trimester. If the karyotype is normal and the follow up scans including expert heart assessment and an anomaly scan in the second trimester do not reveal any features of abnormal development or malformations the chance of good pregnancy outcome is similar to that of the general population[44-46]. Follow-up of children with regards to neuropsychological development has been performed. The two studies which include a control group both report that children with increased NT (≥3.5mm) at the age of 2 years have normal infant development[47;48].

The heterogeneity of the conditions associated with increased fetal NT suggests that the underlying ***etiology*** of the fluid accumulation is multifactorial and is in spite of extensive research not yet fully elucidated. Possible mechanisms include cardiac failure in association with abnormalities of the heart and great arteries[49-51], venous congestion in the head and neck caused by constriction of the fetal body (as seen in diaphragmatic hernia and skeletal dysplasias)[52;53], altered composition of the extra cellular matrix (many of the components are encoded by genes on chromosome 21, 13 and 18)[54;55] and abnormal or delayed development of the lymphatic system[56].

Since the first reports on the association between NT and chromosomal abnormalities a large number of reports have confirmed the association, and thus provided the basis for using NT as an ultrasound marker for chromosomal abnormalities[57]. The initial association studies were quickly followed by prospective interventional studies in specialised centres, in which different NT cut offs were used to describe detection rates and false positives rates in relation to trisomy 21 in a screened study population[57]. The studies showed that measurement of the NT was feasible in > 99% of pregnancies[57]. When combining data from the studies having more than 200,000 screened pregnancies with 871 cases of trisomy 21 it was demonstrated that increased nuchal translucency in combination with maternal age can identify >75% of cases with trisomy 21 for a false positive rate of 5%[57].

Pandya et al. introduced the concept of providing patient specific risk estimates in the first trimester based on maternal age and the NT measurement[58]. By multiplying the maternal age related risk with the LR given by the NT measurement a post test risk specific to the pregnant woman could be calculated. In 1998 Snijders et al. published a large multicenter study coordinated by the Fetal Medicine Foundation in London based on 96,000 pregnancies[59]. The individual patient specific risks were calculated based on maternal age, gestational age and fetal NT thickness. Using

a risk cut off of 1:300 the screening strategy was found to have a detection rate of 77% for a false positive rate of 5%. Figure 7 shows the frequency distributions according to NT deviation in the study to visualise the NT screening potential.

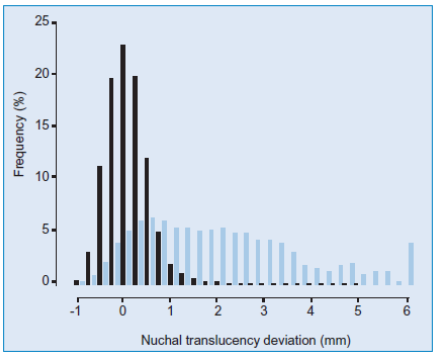

Figure 7: Frequency distributions for NT measurement expressed as deviation from the normal median for CRL in chromosomally normal fetuses (black bars) and trisomy 21 fetuses (light blue bars) (permission from FMF, based on data reported by Snijders et al. 1998)

The high performance was confirmed among others in a study from Denmark. Woejdemann et al. performed a large population based screening study (Copenhagen first trimester study), including 8,995 fetuses with an NT measurement and when using a 1:250 cut off they found a detection rate of 75% for a false positive rate of 1.8%[60].

While gaining increased experience with the first trimester NT measurement in larger datasets, it was found that NT increases with gestational age (as shown in figure 8). Instead of using a fixed cut off at 2.5 mm or 3 mm to define a high risk group it was found that using an NT measurement which was corrected for gestational age (according to measurement of the crown rump length (CRL)) made the performance of the NT better as a marker for chromosomal abnormalities[61]

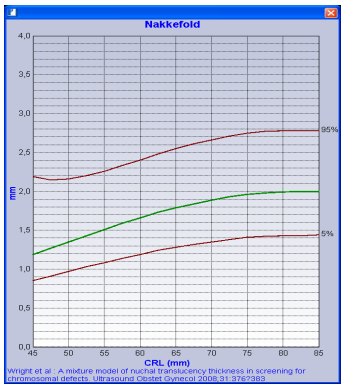

Figure 8: Distribution of NT measurements according to CRL. The green line represents the 50 percentile and the red lines the 5th and 95th percentile (permission from Astraia software gmbh)

Different ways of expressing the NT measurement independently of the gestational age and subsequently use the corrected measurement to provide the LR have been suggested[62;63]. Sahota et al. compared the delta-NT-approach, the multiples-of-the-median-(MoM) approach and the mixture-model-approach and found that none of the suggested methods outperformed the others[64]. The currently used concept in the FMF software programmes is the mixture model described by Wright et al. based on two NT distributions; a CRL dependent and a non- CRL dependent[63]. The 99th percentile for NT is for all gestational ages 3.5mm[59].

Biochemical markers

During pregnancy the placenta synthesizes a wide range of proteins and peptides which are secreted into the maternal serum. In cases where there is a difference in the mean concentration of these placenta products in maternal blood in euploid fetuses and fetuses with chromosomal abnormalities, they can potentially be used as screening variables/biochemical markers for fetal aneuploidy using the concept of Gaussian distributions described in the previous section.

Pregnancy associated plasma protein-A (PAPP-A) is a glycoprotein mainly synthesized in the syncytiotrophoblast cells[65]. PAPP-A is a metalloproteinase which specifically cleaves insulin-like-growth-factor-binding-protein-4 (IGFBP-4). By cleaving the IGFBP-4 which inhibits the insulin-like-growth-factors (IGFs) high levels of PAPP-A are associated with higher levels of IGFs, which play a role in fetal growth[66]. PAPP-A can be detected in maternal serum soon after egg implantation and its concentration increases throughout pregnancy with a doubling time of 3-4 days within the first trimester[67].

Free β-human Chorionic Gonadotrophin (free β-hCG) is a subunit of human Chorionic Gonadotrophin (hCG), a glycoprotein like PAPP-A produced by the syncytiotrophoblast. Free β-hCG as hCG is present in the maternal circulation immediately after implantation and its concentration rises exponentially doubling every 48 hours until week 10 of pregnancy[68]. hCG is essential for the maintenance of the placentation throughout pregnancy and is important for the angiogenesis in the placental spiral arteries in the first trimester[69;70]

Because the levels of PAPP-A and free β-hCG change rapidly during the first trimester of pregnancy, the interpretation of a given concentration is highly dependent on the gestational age at blood sampling. This gestational age dependency can be removed by expressing the observed concentration for each marker as a ratio of the median value observed in a normal pregnancy at the

same gestational age. The new unit is called a ***multiple of the median (MoM)*** [27;31]. The distribution of the MoM values for the biochemical markers in normal and trisomy 21 pregnancies usually follows a Gaussian distribution when the MoMs are log transformed, which is convenient when calculating patient specific risks based on LR for a screening test as described previously[71]. In addition to gestational age a number of other maternal or pregnancy associated factors can affect the serum concentration of the markers (listed in table 2). When using PAPP-A and free β-hCG in prenatal screening programmes these factors also need to be taken into account in order to provide accurate individualised risk assessments[72].

	PAPP-A	free β-hCG
Maternal weight[73;74] **-low maternal weight vs. all** **-high maternal weight vs. all**	 ↑ ↓	 ↑ ↓
Multiple pregnancy[75;76]	↑	↑
Smoking[77;78]	↓	↓
Ethnicity[79] **-Afro-caribbeans and Asians vs Caucasians**	 ↑	 ↑
IVF/ICSI[80]	↓	→
multiparous vs nulliparous[72]	↑	↑
IDDM[81]	↓	→
Female fetal sex[82]	↑	↑

Table 2: Maternal and pregnancy characteristics affecting biochemical markers

In a normal pregnancy the median PAPP-A and median free β-hCG MoM value is per definition 1.0. In pregnancies affected by trisomy 21 the concentration of PAPP-A tends to be lower and free β-hCG to be higher, with values typically around 0.5 MoM for PAPP-A and 2.0 MoM for free β-hCG[83-86]. The MoM values in trisomy 21 pregnancies are not stable across gestation[87]. PAPP-A MoM values in trisomy 21 pregnancies are lower early in the first trimester and thus PAPP-A discriminates better as a marker for trisomy 21, if the maternal blood sample is collected in gestational week 8 compared to week 12[85]. The opposite is found for free β-hCG, which is a better marker at later gestational ages, as MoM values in affected pregnancies are higher later in the first trimester (Figure 9).

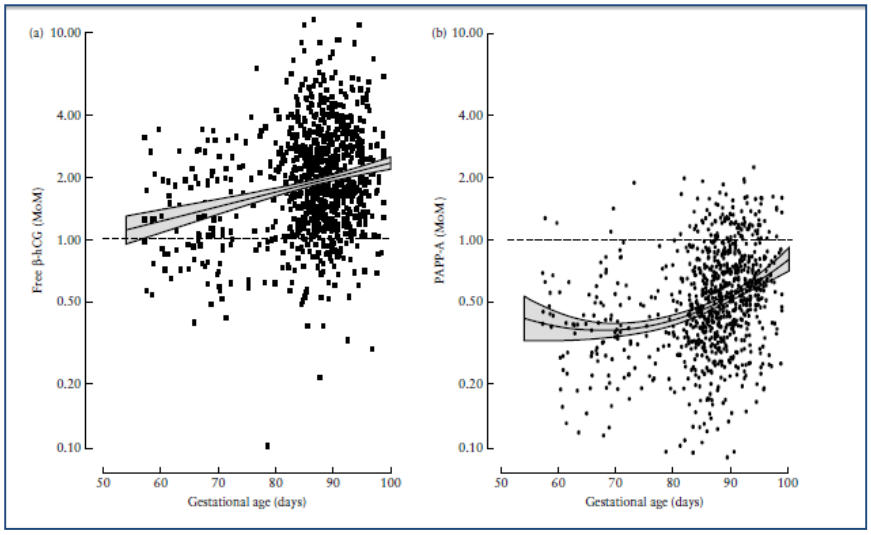

Figure 9: Distribution of Free β hCG MoM and PAPP-A MoM values in pregnancies affected by trisomy 21 according to gestational age with regression lines and 95% CI (Wright et al. 2010)[87]. The discriminatory power for free β-hCG is best after week 12, whereas for PAPP-A the optimal time for blood sampling is before gestational week 10.

Using either PAPP-A or free free β-hCG as a single marker for trisomy 21 in the first trimester in combination with maternal age, PAPP-A has been estimated to have a detection rate of 48-52% for a fixed false positive rate of 5%, while the detection rate for free β-hCG is 42-46%[85;86;88]. In combination the detection rate increases to 67%[86;88]. The performance of the combination of maternal age and the two first trimester biochemical markers has also been confirmed in the Danish first trimester study, where a detection rate of 73% for a false positive rate of 8.8% was reported[60].

As described the maternal serum markers are highly dependent on gestational age. Traditionally ***dating of the pregnancy*** has been performed using information on the last menstrual period (LMP). Dating by ultrasound is a more reliable method, preferably performed in the first trimester of the pregnancy by measurement of CRL[89-92] and it has been found that performance of the screening test for trisomy 21 can be improved by correct dating[93;94].

Combined first trimester screening performance

Association studies and screening studies

The combination of NT and either of the two biochemical markers PAPP-A and free β-hCG all measured in the first trimester of pregnancy was described by Brizot et al. in 1994 and 1995 respectively[83;84]. Spencer and Nicolaides estimated that the detection rate using the combination of

NT, PAPP-A and free β-hCG would be 90% for a 5 % false positive rate[88]. Several other authors have retrospectively estimated a high performance of the combined screening[95-97] and in the beginning of 2000 prospective first trimester screening studies in specialised centres reported detection rates around 90% for a false positive rate of 5 %[57]. In 2005 Nicolaides and co-workers reported the largest prospective screening study performed in seven specialised medical centres on 75,821 pregnant women[98]. They found as expected a detection rate of >90% for a false positive rate of 5.2%. There is thus extensive evidence for the first trimester combined screening strategy to be a reliable screening test for trisomy 21.

Risk algorithms

The complexity of the calculations needed to provide patient specific risk when performing first trimester combined risk assessment has increased steadily during the last decade. Consequently statistical software programmes are required to assist sonographers, biochemists and doctors in calculating risks. Most of the programmes have been developed with support from the Fetal Medicine Foundation (FMF) in London (www.fetalmedicine.com) and the risk algorithms have been developed based on datasets from studies performed primarily in the UK by prof. Nicolaides and his group. They have established a very large database with pregnancy and scanning information on more than 100,000 women, including a large number of cases with chromosomal abnormalities which is essential for development and optimisation of risk algorithms. A recent remarkable outcome of this database has been the development of a mixture model for NT distributions, which quantify the deviation of the measured NT from the expected using two distributions, one which is dependent on CRL and one, that is not[63]. For the biochemistry markers Kagan et al. have recently suggested a multiple regression model to estimate the likelihood ratios for the biochemical markers taking into account the characteristics that influence the measured concentrations of PAPP-A and free β-hCG as described in table 2[72]. In addition a recent paper including Danish data has provided improvement of the algorithm in cases where the biochemical markers are measured early in the first trimester[87]. Validation of the proposed algorithms in independent datasets is an essential task. Kagan et al. reported in 2009 a prospective validation study of the mixture model algorithm for NT measurement and the multiple regression model for biochemistry and thus confirmed the expected performance of the first trimester combined screening using the algorithms suggested by FMF[99].

Population based studies

The majority of data on which the clinical evidence of the screening performance and the algorithms are based, has been collected in highly specialised departments often as part of interventional studies, often in high risk populations. The reason for this is probably that it is not possible to report reliable results from a routine screening set up unless follow-up on pregnancy outcome is done consistently and regularly, as this information is essential when determining detections rates. Follow-up is often time consuming and in many centres impossible, when screening is done in one centre and the delivery in another centre. For that reason there are still only few reports providing evidence on screening performance in routine practice with almost the same screening performance as reported from specialised centres and studies[100-104].

Due to the new national guideline on prenatal screening issued in 2004 Denmark was one of the first countries in the world to routinely offer the first trimester combined screening test to all pregnant women. Our registers in Denmark make national follow-up possible. A national cohort study of the screening performance for the first trimester combined screening programme in Denmark following the new guideline is part of this thesis.

Timing of screening

The best gestational age to perform the first trimester combined screening test has gradually been defined through the development phase over the past 20 years. The optimal gestational age for measurement of the NT is defined by the FMF to be at the time when the fetus has a CRL measurement of 45-84 mm[105], which corresponds to a gestational age of 11+2-14+0 weeks[106]. The reason for selecting 11 weeks as the lower limit for measurement of the NT is that many major fetal malformations such as anencephaly and omphalocele can only be diagnosed after 11 weeks[107;108]. In addition screening necessitates the availability of a diagnostic test. A CVS can safely be performed from week 11. The upper limit of 14 weeks is mainly chosen to provide women with affected fetuses the option of an early and safer termination[105]. Other factors determining the best time for performing the nuchal scan is the fact, that NT is a better marker in the first trimester as the incidence of increased NT thickness in chromosomally abnormal fetuses is lower in the second trimester[36;38], and the finding that the success rate for performing the NT measurement is best in week 10-13[109;110].

The ***OSCAR*** (One Stop Clinic for Assessment of Risk) model, where counselling, NT measurement, blood sampling and risk assessment are provided in one visit around week 12-13 was logistically seen as an advantage when introduced[111]. It was made possible by the development of new

immune assay techniques, which could provide measurement of PAPP-A and free β-hCG within 30 minutes. Recently reports have consistently described improved screening performance for trisomy 21 by measuring the biochemical markers around gestational week 9[72;87;112;113]. In most centres in Denmark biochemical testing and ultrasound scan are carried out at two separate visits, aiming at collecting a blood sample early around week 9-10 and performing the scan around week 12. Theoretically the most optimal timing of the biochemical testing would be to measure PAPP-A early, in gestational week 9 and free β- hCG later, in week 12 due to the above mentioned change in MoM values for trisomy 21 pregnancies dependent on gestational age[87]. Up to now only one paper has investigated how repeated measurement within the first trimester can affect screening performance, examining 261 pairs of samples[114]. A larger prospective evaluation of repeated sampling and its impact on screening performance is presented as paper 3 in this thesis.

Alternative screening models

Modifications of the first trimester combined screening have been proposed. In 1999 Wald et al. introduced the concept of ***integrated testing***, measuring PAPP-A and NT in the first trimester and hCG, AFP, unconjugated estradiol and inhibin A in the second trimester[115]. The result of the first trimester test is not revealed to the pregnant women until the overall test result is ready in the second trimester (week 16-18). They reported a detection rate of 94% for a false positive rate of 5%, which has been confirmed in two large prospective studies[116;117]. This approach has been questioned and has not gained general acceptance as it implies logistic challenges, ethical questions about withholding information and a late termination of pregnancy in affected cases[118].

Another alternative to first trimester combined screening is ***contingent testing***. The principle in contingent screening in general is to divide the screened population into 3 risk groups based on a first stage screening test; a high risk group, which immediately would be offered a diagnostic test, a low risk group which would be given the low risk result and no further testing and an intermediate group, which would be offered a second stage screening test with a re-evaluation of the risk. A number of different contingent screening protocols have been suggested and evaluated in terms of cost/effectiveness[119-127]. Contingent screening within the first trimester is probably the most acceptable approach and was first proposed by Christiansen et al. and Cuckle using either serum biochemistry or NT measurement as the first stage screening test followed by the opposite test in an intermediate risk group[120;121]. Recently Kagan et al. evaluated these two contingent protocols[122].

They report a slightly better performance when using NT measurement as the first stage screening test followed by biochemical testing of only 20% of the population which has an intermediate risk compared to using biochemistry as the first stage screening test. The reported detection rate (90%) and false positive rate (3%) are similar to achievable rates when performing serum biochemistry and NT measurement in all pregnancies. Further improvement of screening performance may be achieved using contingent protocols where first stage consists of a combined first trimester screening test and the second stage is a measurement of additional first trimester ultrasound markers in the intermediate risk group[122;128;129]. This approach is discussed in the fourth paper of this thesis and also in the background section on "new ultrasound markers".

Screening in twins

First trimester screening for chromosomal abnormalities in ***twins*** can be performed by using the NT measurement and the maternal age[130;131]. In dichorionic twins the risk for each fetus is calculated based on the individual NT measurement. The NT thickness is found to be correlated in dichorionic pregnancies which should be taken into account in the risk calculation algorithms[132-134]. In monochorionic pregnancies which per definition are monozygotic the average of the two NT measurements is used in the risk calculation[135]. The serum markers PAPP-A and free β-hCG can be added to improve screening performance but it is essential to adjust for chorionicity and gestational age at sampling[75]. In dichorionic pregnancies the levels of the markers are about twice as high as in singleton pregnancies, while in monochorionic pregnancies the levels are lower than for dichorionic twins[76;135]. The largest twin study performed reports screening performance in dichorionic twins using maternal age, NT measurement and biochemistry to be close to the standard for screening in singleton pregnancies[75].

Screening for other trisomies

As described in the previous section the majority of the first reports which described the association between NT and chromosomal abnormalities were not restricted to trisomy 21 but included all chromosomal abnormalities[57]. As most chromosomal abnormalities are highly associated with high mortality and morbidity they all seem relevant to screen for. When screening moved towards combined first trimester screening introducing the need for complex risk algorithms it became clear, that there was a need for separating the risk algorithms.

Trisomy 18 and trisomy 13 follow trisomy 21 as the most common trisomies[136]. The relative prevalence of trisomy 18 and trisomy 13 to trisomy 21 is 1:3 and 1:7 respectively[57]. Trisomy 18 and trisomy 13 are both associated with increased maternal age and increased NT thickness[58], but there are major differences in the levels of the biochemical markers depending on fetal karyotype. In trisomy 13 and trisomy 18 pregnancies both PAPP-A and free β- hCG are lower than in unaffected pregnancies[137;138]. In addition trisomy 13 is associated with fetal tachycardia[139;140].

Use of the trisomy 21 algorithm will identify around 75% of trisomy 18 and trisomy 13 fetuses[141]. In 2002 Spencer et al. suggested an algorithm for trisomy 13 and trisomy 18[142] and specific algorithms for each trisomy are now available[141;143]. Kagan et al. found that the combination of all three algorithms can detect 95% of trisomy 18 and trisomy 13 fetuses for only a small increase in false positive rate (0.1%) added on the false positive rate given by the trisomy 21 algorithm[141].

Previously the detection of trisomy 13 and 18 was based on detection of anomalies in the second trimester with relatively high detection rates, as a high proportion of these trisomic fetuses have major defects like holoprosenchephaly and exomphalos[144]. As the fetal loss rate during pregnancy is high in fetuses with trisomy 18 and trisomy 13[136], it has been argued that first trimester screening is unnecessary, as the majority of trisomy 18 and trisomy 13 fetuses will either die in utero or be detected by an anomaly scan in the second trimester. The advantage of an early first trimester detection with a possibly safer pregnancy termination seems however to overrule the small increase in number of invasive tests that will have to be performed when screening for trisomy 18 and 13 in the first trimester[145]. The second paper in this thesis evaluates how the change in national screening strategy has affected detection of trisomy 18 and trisomy 13 in Denmark.

Quality control

Screening for trisomy 21 using measurement of the fetal NT and measurement of biochemical markers relies on effective and continuous quality surveillance. A large variation or a consistent bias in the measurement of NT and biochemical markers will lead to significant underperformance of the tests[146-150]. Most biochemical laboratories adhere strictly to internal and external quality control programmes. In Denmark the laboratories are all certified by EN15189 and use the UKNEQAS programme for external quality control (www.UKNEQAS.org.uk). Still it is essential to regularly monitor shifts in MoM values. The FMF software systems provide audit functions also for the biochemical markers. It is also now possible for the laboratories to use own medians when

calculating biochemical MoMs for the risk assessments, as also suggested by Sorensen et al. 2010[151].

Several authors have documented a positive effect on performance when there is intensive focus on training, measurement of quality and follow-up on NT measurement[152-156]. The inter- and intra-observer variation for NT measurements has been reported to be low[157], but dependent on experience of the examiner[158]. It has been estimated that approximately 80-100 supervised scans are needed to achieve a satisfactory repeatability[159]. Recently a new way to minimise operator bias has been reported, where measurement of the NT is performed using a semi-automated system[160;161]. Especially non-expert examiners may benefit from using semi-automated NT measurements[160].

The Fetal Medicine Foundation in London (www.fetalmedicine.com) has been a tremendous resource in organising and centralising training and certification within screening for chromosomal abnormalities. All sonographers performing NT measurements can obtain a certificate of competence by completing an internet based course and submit a logbook of three NT images. In addition it is required to pass a yearly audit consisting of assessment of another three images and an NT distribution curve. The FMF supported software programmes facilitate internal quality assurance on NT distributions. In Denmark it has been decided to fully use the FMF certification and audit system and all sonographers performing first trimester NT measurements are FMF certified.

New biochemical markers

Investigators have tried for many years to supply the market with additional first trimester biochemical markers for trisomy 21[86;162-169]. In a recent meta-analysis Spencer et al. have listed some of the maternal serum biochemical markers and their respective MoM medians in trisomy 21 pregnancies (table 3). By far the best markers are still the free β- hCG and PAPP-A. A new candidate first described in 2003 by Laigaard et al. is ADAM12[167]. ADAM 12 (A disintegrin and metalloprotease) was initially found to be an extraordinarily good marker in the first trimester. Unfortunately, when changing to the second generation laboratory assay, robustness was increased on the expense of considerable loss of discriminatory power[170]. Even though it seems, that some of the biochemical makers might have potential, they are often correlated, and therefore the advantage of adding them to the screening test becomes of limited value[86]. This conventional way of thinking when choosing markers has though been challenged by Wright and Bradbury[171]. They

suggest using repeat measurement of highly correlated markers as an alternative approach for improvement in screening by looking at bivariate distributions of the markers. In addition the future may also bring more studies which examine extremely narrow windows for measurement of the markers within the first trimester as some serum markers' discriminatory power change rapidly with gestational age.

	median MoM	n
PAPP-A	0.45	777
free β- hCG	1.98	846
Inhibin A	1.59	112
Total hCG	1.33	625
AFP	0.80	611
SP1	0.86	246

Table 3: Meta-analysis of published maternal serum markers in cases with trisomy 21 (n) in the first trimester modified after Spencer 2007[86]

New ultrasound markers

In addition to NT, a number of other ultrasound markers have been described in relation to trisomy 21[172]. The development and later the standardisation of the use of the markers have been mainly driven by the Fetal Medicine Foundation. Protocols on how to use the markers and certification processes have been developed and published[172].

The dysmorphic features observed in persons with trisomy 21 has lead to further examination of landmarks in the fetal profile as possible ultrasound markers. The fetal ***nasal bone*** can be visualised on a mid sagittal view of the fetal profile as seen in figure 10. Several studies have demonstrated a high association between absent nasal bone in the first trimester and trisomy 21 and other chromosomal abnormalities[173-176]. Unlike in the second trimester of pregnancy measurement of the length does not seem to add to screening performance of this marker [177;178]. Absence of the nasal bone in the first trimester is found in approx. 60% of fetuses with trisomy 21 and <3% in euploid fetuses (table 4). The prevalence is dependent on gestational age, ethnicity and NT thickness, but independent of the biochemical markers in the first trimester[172] . Adjustment of likelihood ratios is done accordingly using risk algorithms, and performance of first trimester combined screening including the nasal bone is reported to be increased[179] (table 4)

Another facial marker is the ***facial angle***. The image requirement is the same as for the nasal bone, but here even small deviations from the midline may change measurement considerably, and thus 3D ultrasound may aid in performing this evaluation[180]. Increased angle above the 95th percentile is seen more often in fetuses with trisomy 21, and thus accordingly screening performance may be improved by adding this marker into the risk calculation (table 4).

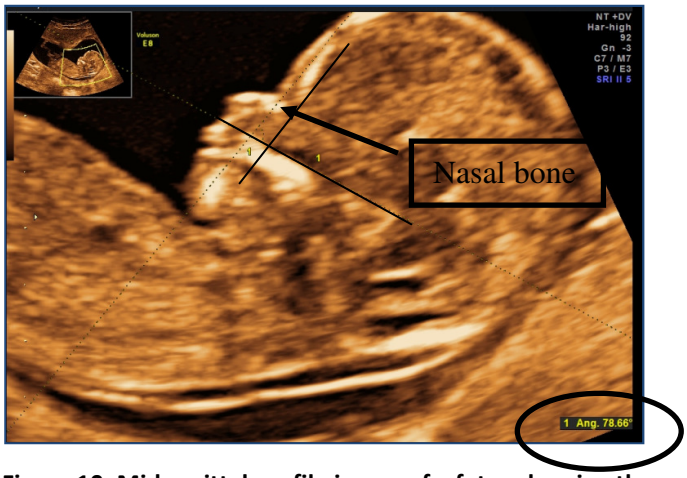

Figure 10: Mid sagittal profile image of a fetus showing the measurement of the facial angle (78.86°, which is normal) and the nasal bone (present).

During the last 5-10 years first trimester assessment of the fetal heart has moved from highly specialised centres to become a possible integrated part of first trimester screening for chromosomal abnormalities as well as screening for cardiac defects[181]. The ductus venosus is a small vessel which in the fetal circulation shunts oxygenated blood from the umbilical vein towards the heart[182]. The ***flow in the ductus venosus*** can be visualised using pulsed Doppler. The normal flow demonstrates forward flow through the cardiac cycle with changes corresponding to various phases of the cycle. Flow during atrial contraction (a-wave) appears to be sensitive to changes in fetal cardiovascular status[172]. The a-wave is normally positive or absent (figure 11), whereas abnormal flow is defined by the FMF as a negative a-wave (reversed flow). As seen in table 4 an abnormal a-wave is more often found in fetuses with trisomy 21 than in euploid fetuses. The reason for this association is not fully understood, however it has been suggested that connective tissue changes in fetuses with trisomy 21 may cause increased stiffness of the myocardial walls leading to an increased right ventricular filling pressure and reversal of the flow through the ductus venosus[172]. The same etiology may be underlying another cardiac marker, tricuspid regurgitation/***abnormal flow across the tricuspid valves***[172]. The described changes in the

connective tissue may result in a relative dilatation of the right ventricle and the right valve annulus[172]. Abnormal tricuspid flow as seen on figure 12 is a more prevalent finding in fetuses with trisomy 21 and thus another possible new ultrasound marker (table 4). Recently Bilardo et al. and Zvanca et al. have both described the finding that ***increased flow in the fetal hepatic artery*** measured by ultrasound pulsed Doppler is associated with trisomy 21[183;184].

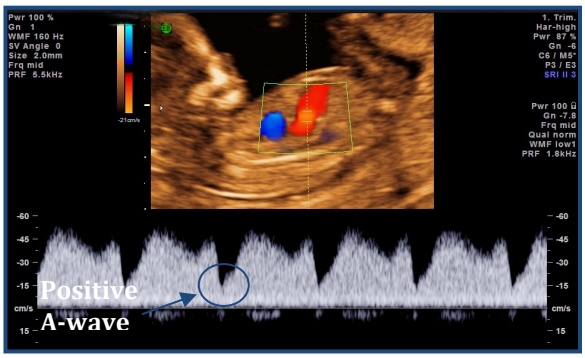

Figure 11 shows a normal flow pattern in the ductus venosus with a positive A-wave

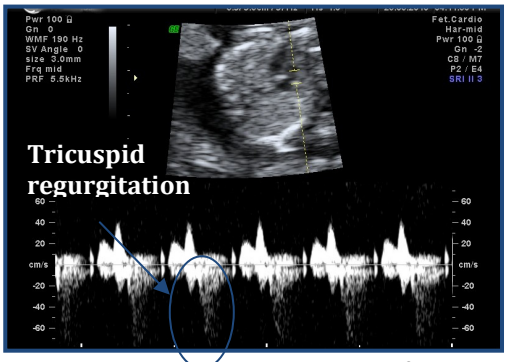

Figure 12 shows abnormal tricuspid flow, with tricuspid regurgitation in the systole of more than 60cm/sec.

Ultrasound markers	Prevalence		Screening performance	
	euploid pregnancies	trisomy 21 pregnancies	DR	FPR
Absent nasal bone[179]	2.6%	59.8%	92%	3%
Increased facial angle > 95 percentile[185]	5,0%	45,0%	92%	3%
Abnormal ductus venosus flow[129]	3.2%	66.4%	96%	3%
Tricuspid regurgitation[128]	0.9%	55.7%	96%	3%

Table 4: Prevalence of the new ultrasound markers and screening performance in combination with NT, PAPP-A and free β-hCG according to FMF studies. (DR=detection rate, FPR=false positive rate)

All the new ultrasound markers should only be used in addition to assessment of the NT, as NT remains the most robust first trimester marker of trisomy 21[186]. As the new ultrasound markers, though in different degree, are dependent on factors like gestational age, smoking, ethnicity and NT thickness it is important to use available risk algorithms provided in the FMF software programmes to get the most accurate individual risk assessment with inclusion of the markers[172]. The risk algorithms are primarily based on large data sets provided in studies supported by FMF. Thus independent validation of the algorithms still seems to be required.

It has been suggested to use the additional ultrasound markers in ***contingent screening protocols within the first trimester*** and thus reserve the assessments to only a minor proportion of the pregnant women[98]. A first trimester combined screening test divides the pregnant women into 3 categories, high (risk >1:50), intermediate (risk 1:51-1:1000) or low risk (risk < 1:1000). Only women in the intermediate risk group (12% of the population) are offered additional assessment by adding the new ultrasound markers. Thus the required training of sonographers and extra scanning time is kept at a minimum[128;129;177]. The corresponding performance has been found to be as effective as if the ultrasound markers were assessed in all first trimester examinations[128;129]. In the last paper in this thesis we investigated the impact on screening performance for trisomy 21 by including the assessment of the ductus venosus flow and tricuspid flow in a two stage screening strategy.

Other advantages of first trimester screening

While screening for chromosomal abnormalities was the initial reason for performing a first trimester ultrasound and biochemical examination of the fetus, it has now been suggested that the first trimester screening test is the most important examination of the fetus during pregnancy in general. In a recent review by Nicolaides the concept of bringing the first trimester examination in focus has been presented by the term “turning the pyramid of prenatal care”[187].

The list of advantages which can be attributed to first trimester screening has been growing during the last 20 years. Initially one of the clear benefits of performing an ultrasound scan during the first trimester of pregnancy was early detection of pregnancy failure[188]. In addition performing a nuchal translucency measurement requires measurement of the CRL as accurate dating of the pregnancy is essential for the screening test. Dating of the pregnancy by ultrasound in the first trimester using CRL is superior to dating by LMP and thus an advantage for women and obstetricians, as correct dating reduces the number of post-term pregnancies and number of inappropriate inductions[189].

Up to recently unsuspected birth of multiples occurred regularly even in developed countries. Screening by ultrasound for anomalies in the second trimester has probably reduced the number, but still determination of chorionicity has to be performed before 15 weeks of gestation[190;191]. Chorionicity is an important predictor for outcome in twins[192-194], and a first trimester scan allows for optimized pregnancy care according to chorionicity[195]. A number of publications have also addressed the possibilities of predicting twin-twin-transfusion-syndrome in the first trimester by assessing the NT thickness and possible CRL discordance in monochorionic twins[196-198]. Thus a first trimester scan is essential in twin pregnancies.

As mentioned briefly in the section on NT, increased NT thickness is related to a wide range of fetal abnormalities and syndromes. NT measurement can be viewed as a screening test for other fetal complications and it is relevant to follow-up on these pregnancies and offer additional ultrasound examinations later in the pregnancy[46]. The specific association between increased NT thickness in the first trimester in fetuses with a normal karyotype and cardiac defects was first described by Hyett et al. in 1996[41]. The risk of a cardiac defect increases with increased NT measurement. The prevalence of cardiac defects is 6 times higher in fetuses with a NT ≥99th percentile than in an unselected population[199]. A meta-analysis reported a detection rate of 31% for a false positive rate of 1.3% for major cardiac defects using NT > 99 percentile as the cut off[200]. Other first trimester ultrasound markers such as abnormal ductus venosus flow or abnormal tricuspid flow are also associated with cardiac defects, and additional ultrasound examinations seem appropriate to offer, if one of these markers is present at the routine first trimester assessment[172;201].

The ultrasound equipment has undergone tremendous improvement during the last 20 years and an increasing number of fetal anomalies have become directly detectable when performing a first trimester ultrasound scan. A basic first trimester scan can identify all cases of body stalk anomaly, alobar holoprosencephaly, exomphalos, gastroschisis and megacystis according to Syngelaki et al.[202]. It is considered a major advantage for the parents to opt for an early and safer termination of the pregnancy in cases where an anomaly, which is lethal or associated with severe handicap, is diagnosed already in the first trimester of the pregnancy.

The latest bullets added to the “advantage list of the first trimester scan” are the pregnancy complications e.g. preeclampsia[203], preterm delivery[204] and intrauterine growth restriction [205] for which it is possible to screen in the first trimester of pregnancy. It is highly relevant to identify high risk pregnancies as early as possible in order to plan intensified follow-up and consider intervention[187]. Screening can be done by having access to maternal history and characteristics,

maternal blood pressure, a first trimester ultrasound assessment including assessment of the placental function and cervical length and the biochemical markers PAPP-A and free β-hCG. Risk is calculated using Bayes theorem and the likelihood principles for screening as outlined previously[187]. Thus the concept used in screening for chromosomal abnormalities has formed the basis for possible major improvements in obstetric care in the future.

Ethical aspects, women's attitude and choice

Ethical considerations and dilemmas are closely related to prenatal screening and diagnosis. As described previously the Danish National Board of Health introduced in 2004 a national consensus on offering first trimester screening for trisomy 21 to all pregnant women in Denmark as one of the first countries in the world. Although the Danish Council of Ethics did question whether the new guideline was ethically acceptable, it was accepted by politicians[206]. In the health care system in general there has during the last 10-20 years been increased focus on information, respect for autonomy and personal integrity. Having access to a first trimester screening service which is based on informed consent enhances the autonomy of pregnant women and is considered ethically obligatory according to Chasen et al., Chevenak et al. and Nicolaides et al. [118;207;208]. During the last 5 years many European countries have started to offer first trimester screening to all pregnant women preceded by information[209]. Some countries still do not have political support to change the prenatal service. In Norway the government does not allow doctors to perform any prenatal screening test for chromosomal abnormalities, unless there is a special indication like age above a certain cut off [210]. Thus women living a little more than 100 km north of Denmark do not have the right to choose a prenatal screening test for trisomy 21. The women's attitude towards being given the offer of first trimester screening has been studied by Müller et al. In the Netherlands the majority of the participating women had a positive attitude towards screening and preferred to have the offer[211].

Spencer and Aitken and de Graff et al. have studied, what kind of screening programme women prefer if given a choice. They both report that the majority prefer first trimester screening compared to second trimester and integrated testing[16;212]. Women who are offered a first trimester screening test (both acceptors and decliners) are less anxious during pregnancy and after delivery compared with those who are not offered screening according to Müller et al.[213]. Around 90% of the Danish pregnant women choose to have a first trimester screening test for trisomy 21[2]. It was

emphasised in the Danish guideline from 2004 that women should be given thorough and neutral information about prenatal screening as a basis for decision making[1]. This ***informed choice*** paradigm is an essential part of the prenatal screening concept. Different definitions of informed choice or informed decision-making exist. They all include at least the following two dimensions: first, the decision should be based on relevant information, and second, it should be consistent with the decision maker's values[214;215]. The first dimension has been studied by Dahl et al., who performed a large Danish population based survey on how well informed the pregnant women were in relation to prenatal screening and diagnostic tests after having made an informed choice about participation or not in the screening programme[216]. They found that women were well informed about the test concept and the main condition screened for. The pregnant women were found to be less knowledgeable about test accuracy and the potential risk of adverse findings other than trisomy 21. The same group also investigated how level of knowledge was related to level of decisional conflicts and levels of wellbeing[217]. They found that higher knowledge was associated with less decisional conflict when deciding whether to participate in the first trimester screening programme. In addition high levels of knowledge were associated with increased level of wellbeing. The same conclusions have been reported by other authors[215;218]. Van den berg et al. found in a Dutch study where they examined both dimensions of the informed choice that 68% of the participating women made an informed choice based on both sufficient knowledge and on consistency with the decision maker's values[215].

Individual decision making in prenatal screening is reported to be influenced by social, psychological, cultural, religious and ethical beliefs[219]. Thus it can be a major challenge to provide counseling in prenatal screening. It is essential that health care professionals are aware of their responsibility of giving neutral and thorough information to the pregnant women. Focus has to be kept on offering and not suggesting. Optimally, the health care professional should be using a non-directive counseling philosophy where the client's values are discussed and the counselors views are deliberately excluded[220]. In the Danish Guideline the importance of presenting all treatment possibilities and achievable help provided by the society is emphasized. Counseling by non health care professionals is also possible and information about this should always be given to the couple[1]. In addition it seems essential that the society can offer high standard care during pregnancy and after delivery also to infants with chromosomal abnormalities and to their families.

Epidemiological research in Fetal Medicine

In Denmark we have unique opportunities for performing quality research in Fetal Medicine. As described we have national consensus regarding prenatal screening offers, thus there is a basis for national population based studies. In addition everyone in Denmark is given a unique personal identification number at birth, which is used in all contacts with the social system and hospital system. We use this in our hospital records and registers and it enables us to link information between registers. When the new national guideline was introduced in 2004 all departments in Denmark agreed to use the same software system for risk calculation and storage of ultrasound data; Astraia (Astraia software gmbh, Germany, www.astraia.com). The system is one of the software solutions supported by FMF. Relevant pregnancy and screening data are entered by sonographers or doctors locally. The system provides an audit module for first trimester screening data (NT measurements and maternal biochemistry) and in addition access by a query function to all entered data.

One of the essential parameters when assessing screening performance is detection rate. Complete follow up regarding fetal karytotype is required to calculate a valid detection rate. In Denmark all Departments of Clinical Genetics forward results on all karyotype analyses to the Danish Central Cytogenetic Register which provides central access to chromosomal follow-up.

In Denmark we have established a ***Danish Fetal Medicine Database***[221]. The database is based on data from all 15 local Astraia servers in Denmark and contains data back from 2008. Data entered as part of the routine screening practice when performing nuchal translucency scans and anomaly scans are sent daily to a common server. Before the national database was started all departments in Denmark agreed to use the same standard curves for biometries in the first and second trimester[222]. It was also decided to use CRL measurement in the first trimester to estimate date of delivery. A 1:300 risk is used as a cut off point for referral to invasive diagnostic testing[223]. In addition a set of mandatory data for each scan type was defined. Outcome data of the pregnancy is collected from the Danish Central Cytogenetic Register (prenatal and postnatal chromosome analyses), the National Patient Register (spontaneous and induced abortions) and from the National Birth Register, from where information about pregnancy, delivery and the newborn is received. All this information is linked in the Danish Fetal Medicine Database using the unique personal identification number. For each pregnancy, scanning information on one or more fetuses is linked to a karyotype result if performed during or just after the pregnancy, and in addition either

information on loss of pregnancy or delivery is available for every pregnancy (figure 13). Data from the national database can be used for local and national quality assessments as well as for research projects.

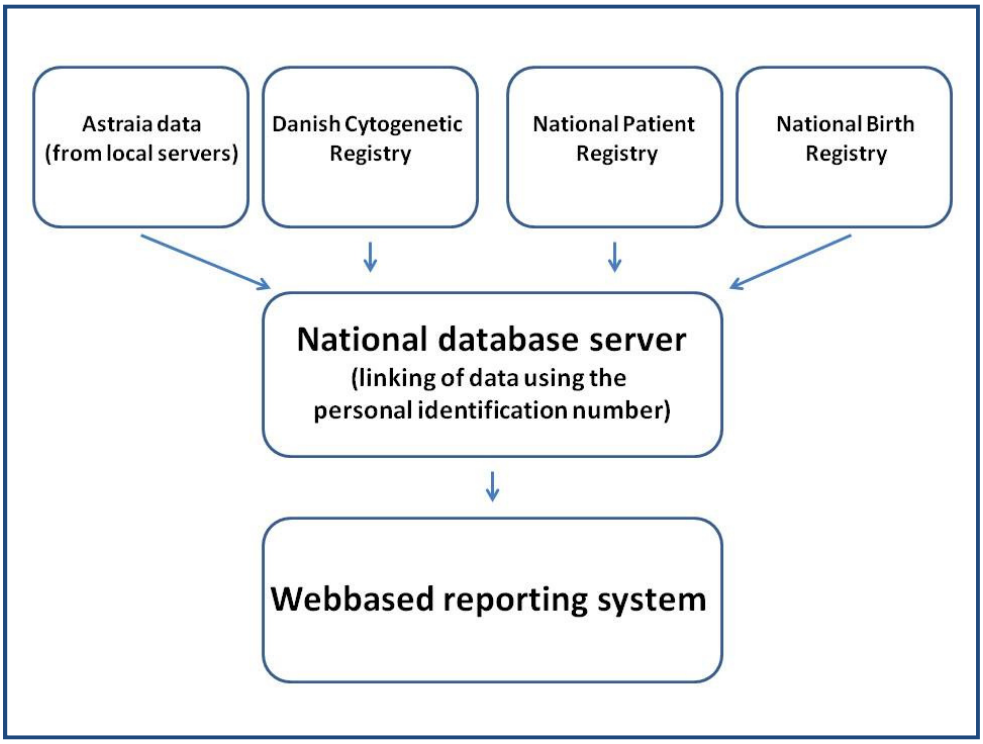

Figure 13: Data sources in the Danish Fetal Medicine Database

Own studies

Paper 1

Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study.

Aim

Our aim was to evaluate the impact of a new national first trimester screening strategy introduced in Denmark in 2004 on the number of Down's syndrome births and the rate of chorionic villus samples and amniocenteses. In addition we wanted to determine detection and false positive rates in the screened population in 2005 and 2006.

Methods

It was a population based cohort study. Pre- and postnatally detected cases of Down's syndrome and the number of invasive diagnostic procedures were retrieved from the Danish Central Cytogenetic Register for the period 2000 to 2007. Data was collected from all 19 departments of obstetrics and gynaecology in Denmark performing the first trimester risk assessment. Information on number of women screened in 2005 and 2006, screen positive rate and screening information on postnatally diagnosed infants with Down's syndrome were retrieved.

Results

First trimester combined risk assessment was introduced successively. In January 2005 nine out of 15 counties (60%) offered the assessment and by June 2006 the offer covered the entire country.
The number of newborns with Down's syndrome was reduced from 55-65 per year in 2000-2004 to 31 and 32 in 2005 and 2006 respectively.
The total number of prenatal diagnostic procedures (chorionic villus samples or amniocenteses) decreased from 7,524 in 2000 to 3,510 in 2006. The proportion of chorionic villus samples increased from 44% to 66% during the same period.

Approximately 65,000 women were pregnant in Denmark in each of the years 2005 and 2006. In 2005 40,815 women (approx. 63%) had a first trimester risk assessment performed, while this number rose to 54,830 (approx. 84%) in 2006. In 2005 a total of 1,706 women (4.2%) had a risk $\geq$ 1:300 (screen positive rate) while this number was 1,899 women in 2006 (3.5%).

In the population screened in 2005 the detection rate of Down's syndrome was 86% [95% CI 79-92%] as 104 were screened true positive out of 121, who carried a Down's syndrome fetus. In 2006 the detection rate was 93% [95 % CI 87-97%] as only 7 women received a false negative screening result.

Discussion

To the best of our knowledge this is the first report describing the impact of a nationwide offer of a first trimester combined risk assessment for Down's syndrome. Even before the policy was fully implemented, a dramatic effect could be seen, as the number of children born with Down's syndrome decreased by approximately 50%, and the number of prenatally detected cases increased by around 30 %. We also found, that the number of prenatal diagnostic tests (chorionic villus samples and amniocenteses) performed yearly decreased by more than 50 % from 2000 to 2006.

We found detection rates for Down's syndrome of 86% and 93% respectively in the screened populations in 2005 and 2006 for false positive rates of 3.9% and 3.3%. This is in accordance with the screening performance expected by the National Board of Health when it was decided to implement this new screening strategy. This performance may be considered very high, especially since the screening programme in 2005 and 2006 in many centres implied a completely new screening method. Furthermore we report the result of routine clinical practice, where not all risk assessments are based on the optimal parameters (combination of maternal age, nuchal translucency scan and biochemical test) as some are given only on maternal age and nuchal translucency or biochemistry. Other authors have also reported screening results achieved in routine clinical practice in up to 13 centres with detection rates between 83% and 93% and false positive rates between 3.9% and 5.9%.

Our data show that it is possible to introduce this screening strategy in as many as 19 different centres and still obtain national detection and false positive rates similar to those from specialized centres. It is well known that the implementation of new screening strategies requires extreme efforts. In Denmark with a public, free-of-charge hospital system, we have succeeded in establishing a strong national fetal medicine organisation. Recruitment and training of sonographers as well as quality control is in accordance with the guidelines from the Fetal Medicine Foundation in London (www.fetalmedicine.com). We have implemented national guidelines on first trimester screening and from 1 January 2007 a common cut-off for referral to invasive diagnostic testing of 1:300 at the time of screening has been implemented. Furthermore the use of the same database software in all departments allows for national data merging. A national quality database has been established, merging data from all fetal medicine units, the Danish National Cytogenetic Register and the National Patient Register. This will allow national follow on up on all screened women and monitoring of national detection rates and false positive rates, as well as invasive testing rates, a quality control which is considered essential following the implementation of a new screening programme.

Paper 2

First-trimester screening for trisomy 21 in Denmark: implications for detection and birth rates of trisomy 18 and trisomy 13

Aim

The aim of this study was to investigate whether the new screening strategy for T21 has changed the gestational age at which trisomy 18 (T18) and trisomy 13 (T13) are diagnosed prenatally, and changed the number of infants born with T18 or T13.

Material and methods

From the Danish Central Cytogenetic Register we collected information on all prenatal and postnatal chromosome analyses for T18 or T13, registered from 1997 to 2007. Information on first-trimester screening results was collected from each department of obstetrics and gynecology performing the nuchal translucency scans. The cut-off used at screening for referral to invasive diagnostic testing for T21 and for T18/T13 was 1 : 300 and 1 : 150, respectively.

Results

In total, there were 435 cases with T18 and 168 cases with T13 between 1997 and 2007 in Denmark. The estimated incidence of T18 and T13 at the time of delivery was calculated as 2.5 and 1.6 per 10,000 deliveries, respectively. The number (proportion) of cases diagnosed before week 18 increased significantly, from 63 (59.4%) in 1997 and 1998 to 90 (80.4%) in 2006 and 2007 ($P < 0.001$). In addition, the number of T18 and T13 cases diagnosed prenatally after week 22 or postnatally decreased significantly, from 34 (32.1%) in 1997 and 1998 to seven (6.3%) in 2006 and 2007 ($P < 0.0001$). For women participating in first-trimester risk assessment in 2006 and 2007, the detection rate of T18 and T13 was 78.8% (95% CI, 71.0–86.7%).

Discussion

This report describes how the recently introduced national guideline on first trimester screening for T21 used in routine clinical practice has changed the detection of T18 and T13. We found a significant increase in the number of T18 or T13 fetuses diagnosed before week 18 and a significant decrease in the number of infants born with T18 or T13. Most importantly, the introduction of the new screening strategy has also resulted in a marked decrease in the number of diagnostic invasive tests. From 2000 to 2006 the yearly number of invasive diagnostic procedures in Denmark thus decreased by more than 50%.
Several authors have reported performance of the first trimester combined screening for T18 and T13. In 2002 Spencer and Nicolaides derived a combined risk algorithm for T18/T13 with a predicted detection rate of 95% using the risk cut off of 1:150. We found a first trimester detection rate of T18 and T13 of 78.8%

using the same algorithm and cut off. Overall, it seems that detection rates observed in series prospectively collected are not as high as those expected based on modelled data sets. In a large prospectively collected Danish cohort, Kirkegaard et al. reported a 73% detection rate of T18 or T13 (cut off 1:150 for T18/T13, 1: 300 for T21).

One could argue that screening for T18 and T13 in the first trimester is not necessary, as the majority of fetuses will either die in utero or be detected if a second trimester scan is performed. Detection rates for T18 and T13 in the second trimester by ultrasound scan have been reported to be 80-86% for T18 and 90-100% for T13. However, no algorithm for the second trimester sonographic screening is available and the screen positive rate is not known. As the overall screen positive rate only increases slightly (0.1%) when including the screening algorithm for T18 and T13 in the first trimester screening programme for T21, it is considered acceptable to include screening for T18 and T13 in the screening programme. Most women prefer screening to be performed early in pregnancy and should the fetus be diagnosed with T18 or T13, the parents can opt for a safer termination of the pregnancy in the first trimester.

The main strength of our study is that we have a large national data set collected in a homogenous low risk population. Local quality assurance of the ultrasound scans are performed via the Fetal Medicine Foundation (www.fetalmedicine.com). The biochemistry departments are accredited by EN15189. Our national cytogenetic register compiles results from all chromosome analyses and is considered to be almost 100% complete. Our prenatal screening information on cases with T18 or T13 in 2006 and 2007 is considered complete, as we were able to find detailed screening information on all cases registered in the Danish Central Cytogenetic Register.

A limitation of our study is that, although the data were registered prospectively, our main objective was to retrospectively look at the gestational age at which T18 and T13 were diagnosed throughout the 11-year period. Therefore, we do not have information on the overall first trimester screen positive rate for T18/T13. We do, however, not expect it to vary from the very low false positive rates (< 0.5%) expected and reported in other study populations.

Paper 3

A prospective study evaluating the performance of first trimester combined screening for trisomy 21 using repeated sampling of the maternal serum markers PAPP-A and free β-hCG

Aim

The aim of this study was to prospectively evaluate the screening performance of first trimester combined screening for trisomy 21 using a double set of the biochemical markers PAPP-A and free β-hCG

Material and methods

Three Fetal Medicine departments in Denmark participated in the study. Screening for trisomy 21 was set up as a two-step approach with blood sampling performed before the nuchal translucency scan (early sample). A second blood sample was collected at the time of the nuchal translucency scan (late sample). PAPP-A and free β-hCG were measured on both the early and the late samples.

We defined *four* first trimester screening protocols. All protocols used maternal age, nuchal translucency thickness and biochemical markers in different combinations. Protocol 1 used both PAPP-A and free β-hCG from the late sample, protocol 2 used both PAPP-A and free β-hCG from the early sample, protocol 3 used PAPP-A from the early and free β-hCG from the late sample and protocol 4 used both markers from both samples. Auto and cross correlation between the markers were determined and age standardised detection and false positive rates for the different screening protocols were estimated.

Results

We collected two blood samples in 27 pregnancies affected by trisomy 21 and in 3891 control pregnancies. The early samples were taken between gestational week 8+0 up to week 13+6, and the late samples between week 11+3 up to week 14+6. The median interval between the samples was 17 days (range 1-40 days).

We calculated screening performance according to the four screening protocols for different fixed cut-offs. We found a significantly better ($P<0.05$) estimated screening performance when using early sampling vs. late sampling (protocol 2 vs. protocol 1). With a risk cut-off of 1 in 100, at the time of the risk assessment, the estimated detection and false positive rates were 91% (95% CI: 81-98%) and 1.6 % (95% CI: 1.3-2.0%) respectively when the blood sample was collected early (protocol 2). Better estimated performance was achieved with the use of the double set of markers (protocol 4); detection rate 93% (95%CI: 85-99%) and false positive rate 1.7% (95% CI 1.4-2.0%), but this was not significantly different from the early sample protocol (protocol 2) ($P>0.5$).

Discussion

This is the first large prospective study performed to investigate the potential benefits of having access to a double set of maternal serum markers within the first trimester of a pregnancy.

We found that screening performance is improved when risk calculation is based on an early first trimester sample compared to the performance found when the sample is taken at the time of the nuchal translucency scan. This finding was expected due to previously published data on the biochemical markers' gestational age dependent discriminatory power and in line with two previous studies (Kirkegaard et al. and Borrelle et al.), which evaluated early blood sampling in the first trimester.

One paper has previously investigated if repeat maternal serum screening within the first trimester can improve screening performance. Spencer and Cuckle used a data set with 261 paired samples taken between weeks 10+0 to 13+6 from unaffected pregnancies. They found a detection rate of trisomy 21 of 88.6% for a fixed false positive rate of 5%, which was only a 1.3% increase in detection rate compared to using only one sample. When using this large dataset including 27 cases of trisomy 21, we found the screening performance to be very high (detection rates 97% for a false positive rate of 3%) when using two samples (protocol 3 or 4).

The strength of our study is that it is a large study with prospectively collected data including trisomy 21 cases, and the presented results are not based on modelled data. The data set was collected at three different centres with slightly different ways of handling the samples. Thus the data do not represent a strict study set up, and therefore we believe that our study results reflect what is probably achievable in routine clinical settings. Although the data set does contain cases with trisomy 21, the number is still limited. It is possible that the correlation matrixes and the risk calculations can be improved by having access to more data. Thus before repeated testing is offered to pregnant women outside a study set up we do suggest validation of the results in independent data sets.

In conclusion we have described how the performance of the first trimester combined screening programme is affected by the time of blood sampling. Having access to an early blood sample is superior to sampling at the same time as the nuchal translucency scan. In addition it seems possible to improve the screening performance even more, if repeated blood sampling within the first trimester is performed. Optimisation of screening performance in a screening programme like first trimester combined screening for trisomy 21 which is used world wide is always relevant as even small improvements may lead to a substantial decrease in the number of invasive tests performed and subsequently in number of pregnancies affected by procedure related loss. This should however be weighed against the fact that a number of women will refrain from screening if the procedure is too complicated.

Paper 4

Screening performance for trisomy 21 comparing first trimester combined screening and a first trimester contingent screening protocol including ductus venosus and tricuspid flow.

Aim

Our aim was to to compare the standard first trimester combined risk assessment for trisomy 21 with a contingent screening protocol including tricuspid flow and ductus venosus flow.

Material and method

The study was a two centre prospective study. Singleton women with a risk assessment > 1:1000 were included and had additional assessment of the ultrasound markers. We compared screening performance in two screening strategies; a) First trimester combined screening strategy based on the individual risk results from the routine screening test. b) A contingent screening strategy based on a combination of the routine test results and additional ultrasound markers.

Results

A total of 23 cases with trisomy 21 were included in the study population (trisomy 21 group) and 894 pregnancies with a known normal prenatal karyotype or with no registered abnormal karyotype postnatally (euploid group).

The routine *first trimester combined screening strategy* had in our high and intermediate risk study population an overall detection rate of 100 % (23/23), as all cases with trisomy 21 in the study population had a combined risk assessment $\geq$ 1:300. A total of 48.3% (443/917) had a risk at or above 1:300 based on the combined screening test where tricuspid flow and ductus venosus flow were not taken into account in the risk assessment.

Using a *contingent screening strategy*, where screen positives are defined as having either a risk assessment $\geq$ 1: 50 or a risk assessment between 1:51 and 1:1000 and either abnormal tricuspid and/or abnormal ductus flow lead in our study to a significant decrease in screen positive rate from 48.3% to 17.7% ($p< 0.001$). We found a decrease in detection rate from 100% to 91.3% (21/23), but this was not statistically significant ($p=0.48$).

Discussion

We found that inclusion of the markers in a contingent screening set up can significantly reduce the number of women who are screened positive by the first trimester combined screening strategy. This is in accordance with a number of reports from similar studies. In the majority of the published work the study populations are small sub populations often with high risk for trisomy 21. The two major studies from Fetal

Medicine Foundation however provide good evidence for the rationale of assessing additional markers in a contingent screening set up, with only about 15% of the total population needing assessment of these additional markers. Kagan et al. and Maiz et al. found that inclusion of the additional markers would, in a low risk population result in a detection rate of 96% for a screen positive rate of 3%. If our population based screen positive rate was fixed at 5%, our total population size would have been 8860 women (443/8860= 5%). Using a contingent screening strategy the screen positive rate would be 1.8% (162/8860) and the detection rate 91.3%. These results are very similar to those found by Maiz et al. and Kagan et al.

The study was performed with a different set up in the two participating centres. As one centre was strictly investigator driven, this centre provided a dataset where almost all participants had assessments of tricuspid and ductus venosus flows. The other centre provided data from a set up where assessments of the flows were performed as part of routine practice although results of the additional markers were not included in the routine risk assessment. In the routine screening set-up, where 30 minutes are provided to perform scanning and counselling, sometimes only one of the flows was recorded or neither of them were assessed in the women eligible for inclusion. This could potentially have affected the results. Since our results are in agreement with results published by other groups, however such a possible effect does not seem to be of any major importance.

In Denmark the first trimester combined screening programme, which is offered to all pregnant women, is reported as well implemented using the first trimester combined screening strategy with high screening performance. Our suggestion would be to continue a prospective collection of data on additional ultrasound markers and continue to train sonographers in the assessment of the flows. Further studies performed in routine clinical settings which provide validation of the available algorithms are needed before we would consider using the markers routinely in our national screening programme.

Conclusion and future perspectives

The aim of this thesis was to evaluate the first trimester combined screening programme for trisomy 21 in Denmark implemented following the new national guideline in 2004. We found that the majority of pregnant women in Denmark do have a first trimester combined screening test performed. We also found that the screening performance in our national programme does meet the expectations from other prospective validations reported on the first trimester screening test. In addition the programme has, as expected, resulted in an increase in number of fetuses with trisomy 18 and trisomy 13 being diagnosed early in pregnancy.

It is essential to perform quality assurance regularly when running a screening programme like we have in Denmark. Local quality assurance includes surveillance of NT distributions, biochemical MoM distributions and screen positive rate, and fortunately this is quite easily done using the developed computer programmes, which is usually available in centres performing the screening. Calculating detection rates require follow-up on all screened fetuses during the rest of pregnancy and after delivery. It is time consuming and in some centres impossible. In the studies presented here on national data we used the local screening information and national cytogenetic register information and were able to link data using the personal registration numbers. It did require some effort to collect relevant data and after we performed the studies a national database has been established with an almost automatic collection of screening and outcome data at a national level. Thus a follow-up evaluation of the Danish National Screening programme based on data from the Danish Fetal Medicine Database is expected in the near future. We anticipate this database to become a unique and powerful tool within fetal medicine research, not only in relation to screening for the most common chromosomal abnormalities, but also in relation to screening for uncommon chromosomal abnormalities and other adverse pregnancy outcomes.

In the future screening for chromosomal abnormalities may be unnecessary, if a non-invasive diagnostic test becomes available. It is difficult to know when this will be commercially offered and therefore it is still important to look for new ways of improving the current screening strategies for chromosomal abnormalities. Another aim of this thesis was to assess two possible new screening strategies for trisomy 21. We found that screening performance can be improved by optimised timing of blood sampling and repeating the blood sampling within the first trimester. We also investigated the improvement in screening performance using a contingent screening strategy and two new ultrasound markers. We found that by using this screening strategy it would be possible to lower the screen positive rate significantly. The screen positive rate is directly related to the

number of invasive tests and thus it is important to keep it as low as possible to avoid unnecessary fetal losses. It is now evident, that different new screening strategies are promising in terms of lowering the screen positive rate. It is indeed relevant to investigate and improve the screening using different strategies, as fetal medicine centres around the world do differ in how the service is set up and in available resources. As screening for trisomy 21 becomes more and more common also in less developed countries with limited number of sonographers and possible logistic challenges in collection of blood, it is relevant to optimise and develop different contingent screening programmes in order to use existing resources best.

During the last 30-40 years screening for chromosomal abnormalities has changed from using maternal age as a single screening variable to using multiple markers and complicated risk algorithms which can provide specific individual risk assessments within the first 3 month of the pregnancy. It has become an available offer in many countries and a major advantage for many pregnant women and their fetuses as this screening strategy reduces the number of fetal losses due to unnecessary invasive procedures. In the future it may become more common also to screen for other complications in pregnancy such as preeclampsia and preterm delivery using the concept developed in screening for chromosomal abnormalities. Still it should be emphasised that also in the future screening for adverse pregnancy outcome as well as for chromosomal abnormalities should always be based on an informed choice where women on the basis of knowledge and values make individualised decisions and are free to accept or decline screening in pregnancy.

Summary in English

The main topic of the thesis is prenatal screening for trisomy 21. The thesis can be divided into 3 parts.

Part one:

The first part consists of two epidemiological studies performed to evaluate the impact of a new national screening strategy for trisomy 21 in Denmark. In the ***first study*** our objective was to evaluate the nationally implemented first trimester combined screening programme for trisomy 21. Information was collected information from the Danish National Cytogenetic Register from 2000 to 2007 on the number of pre- and postnatally detected cases of trisomy 21 and number of invasive diagnostic procedures. In addition data on screening information from 2005 and 2006 from all departments in Denmark performing first trimester screening was collected. We found the number of newborns with trisomy 21 to be significantly reduced after the introduction of the new screening policy. We also found a sharp decline in the number of prenatal invasive procedures performed throughout the country. We concluded that our national screening performance based on calculated detection rates and false positive rates in 2005 and 2006 was similar to performance reported from single specialised fetal medicine centres.

In the ***second study*** we aimed at assessing, whether the new national screening programme for trisomy 21 had changed the gestational age at which trisomy 18 and trisomy 13 are detected. We collected information from the Danish National Cytogenetic Register on all prenatal and postnatal chromosome analyses for trisomy 18 or trisomy 13, registered from 1997 to 2007. We also collected information on first-trimester screening results from each department of obstetrics and gynecology performing the nuchal translucency scans. The number of trisomy 18 and trisomy13 fetuses diagnosed before week 18 had significantly increased after the introduction of a combined first-trimester screening strategy for trisomy 21 in Denmark. In addition and consequently, the total number of fetuses diagnosed late in pregnancy and infants born with trisomy 18 or trisomy 13 had decreased significantly.

Part two:

This part of the thesis consists of a prospective study (***third study***) performed in collaboration with Hvidovre Hospital and Aarhus University Hospital, Skejby. Our objective was to investigate if access to a double set of the maternal serum markers Pregnancy Associated Plasma Protein-A (PAPP-A) and free β-human Chorionic Gonadotrophin (free β-hCG) could improve screening performance for trisomy 21. We collected an additional blood sample from 3918 women, who came for their nuchal translucency scan and who prior to this scan had had a blood sample taken as part of the screening test. We measured the biochemical markers PAPP-A and free β-hCG in both samples, and thus we had access to two serum marker samples from the same pregnancy. We found that repeated blood sampling with measurement of PAPP-A and free β-hCG in the combined first trimester screening could optimise screening performance for trisomy 21. The detection rate was increased from 92% to 97% for a fixed false positive rate of 3% when comparing first trimester

combined screening performance based on one blood sample taken at the same time as the nuchal scan with the performance where two blood samples were available for risk calculation.

Part three:

This was a prospective study (***fourth study***) performed in collaboration with Aarhus University Hospital, Skejby. Our aim was examine two new ultrasound markers for trisomy 21 (abnormal ductus venosus flow and tricuspid regurgitation) in a Danish cohort, and evaluate their possible impact on screening performance. We included 917 singleton pregnant women with a risk assessment > 1:1000 based on their routinely performed first trimester combined screening test. There were 23 cases of trisomy 21 in the study population. We measured ductus venosus flow and/or tricuspid flow in relation to the nuchal translucency scan. We found that inclusion of the markers in a contingent screening set up can significantly reduce the number of women who are screened positive by the first trimester combined screening strategy. This is in accordance with a number of reports from similar studies.

Dansk resume

Afhandlingens overordnede emne er prænatal screening for trisomi 21 i Danmark.

Afhandlingen kan inddeles i 3 dele.

Første del:

Den første del består af to epidemiologiske studier udført for at evaluere virkningen af en ny national screeningsstrategi for trisomi 21 i Danmark. I det ***første studie*** var formålet at evaluere det danske første trimester screeningsprogram for trisomi 21 på nationalt plan. Oplysninger fra Dansk Cytogenetisk Centralregister for perioden 2000 til 2007 vedrørende antallet af præ-og postnatalt diagnosticerede tilfælde af trisomi 21 og antallet af invasive diagnostiske procedurer blev indsamlet. Derudover indsamledes screeningsoplysninger fra 2005 og 2006 fra alle afdelinger i Danmark, som udfører første trimester screening. Vi fandt, at antallet af børn født med trisomi 21 var blevet væsentligt reduceret efter indførelsen af den nye screeningspolitik, og at antallet af prænatale invasive procedurer var halveret. Vi fandt desuden, at vores nationale screeningsperformance baseret på udregnede detektionsrater og falsk positive rater i 2005 og 2006 svarede til det forventede og var på samme høje niveau, som performance rapporteret fra højt specialiserede, mindre føtalmedicinske afdelinger i udlandet.

I det ***andet studie*** var vores formål at undersøge, hvorvidt det nye nationale screeningsprogram for trisomi 21 havde ændret gestationsalder ved diagnose af trisomi 18 og trisomi 13. Vi indsamlede fra Dansk Cytogenetisk Centralregister oplysninger om alle prænatale og postnatale kromosom analyser med fund af trisomi 18 eller trisomi 13 registreret fra 1997 til 2007. Vi indsamlede ligeledes oplysninger om første trimester screeningsresultater fra alle afdelinger i Danmark, som udfører første trimester screening. Antallet af trisomi 18 og trisomi 13 fostre diagnosticeret før graviditetsuge 18 var signifikant øget efter indførelsen af en kombineret første trimester screeningsstrategi i Danmark. Tilsvarende fandt vi, at det samlede antal fostre, der bliver diagnosticeret med trisomi 18 eller trisomi 13 sent i graviditeten, eller som bliver født, var faldet signifikant.

Anden del:

Denne del af afhandlingen består af et prospektivt studie (***tredje studie***) udført i samarbejde med Hvidovre Hospital og Århus Universitetshospital, Skejby. Formålet var at undersøge, om adgang til et dobbelt sæt af moderens serum markører Pregnancy Associated Plasma Protein-A (PAPP-A) og frit β-humant Chorion Gonadotropin (frit β-hCG) kan forbedre screeningsperformance for trisomi 21.

Vi indsamlede en ekstra blodprøve fra 3918 kvinder, der kom til nakkefoldskanning, og som forud for denne havde fået taget en blodprøve med måling af serum markører som en del af rutine-screeningen for trisomi 21. Vi gentog målingen af PAPP-A og frit β-hCG i den ekstra blodprøve og havde således adgang til et dobbelt sæt serum markør-prøver per deltager. Vi fandt, at først trimester screeningsperformance i relation til

trisomi 21 kunne forbedres ved at anvende 2 sæt tidsmæssigt adskilte blodprøver med bestemmelse af PAPP-A og frit β-hCG i begge prøver. Detektionraten steg fra 92% til 97% for en fixeret falsk positive rate på 3%, når man sammenlignede med screeningsperformance opnået ved anvendelse af en enkelt blodprøve taget samtidig med nakkefoldsskanningen.

Tredje del:

Dette var et prospektivt studie (***fjerde studie***) udført i samarbejde med Århus Universitetshospital, Skejby. Formålet med dette studie var at undersøge forekomsten af to nye ultralydsmarkører for trisomi 21; abnormt ductus venosus flow og abnormt flow over tricuspidalklapperne i en dansk kohorte, og evaluere deres mulige indvirkning på screeningsperformance. Vi inkluderede 917 singleton gravide med en risikovurdering > 1:1000 baseret på deres rutinemæssigt udførte første trimester screeningstest. Der var 23 tilfælde af trisomi 21 i den undersøgte population. Vi målte ductus venosus flow og/eller tricuspidal flow i forbindelse med nakkefoldsskanningen. Vi fandt, at det er muligt at forbedre risikovurderingen for trisomy 21 ved at inkludere de to markører. Dette er i overensstemmelse med resultater fra lignende internationale studier.

References

(1) The Danish National Board of Health; Guideline on prenatal information, screening and diagnosis 2004 *http://www.sst.dk/publ/Publ2004/Informeret_valg.pdf. 2004.*
Ref Type: Internet Communication

(2) Ekelund CK, Andersen HJ, Christensen J, Ersbak V, Farlie R, Henriques C et al. [Down's syndrome risk assessment in Denmark--secondary publication]. Ugeskr Laeger 2010; 172(23):1759-1761.

(3) Megarbane A, Ravel A, Mircher C, Sturtz F, Grattau Y, Rethore MO et al. The 50th anniversary of the discovery of trisomy 21: the past, present, and future of research and treatment of Down syndrome. Genet Med 2009; 11(9):611-616.

(4) Vogel I. Prænatal diagnostik på klinisk genetiske afdelinger. In: Tabor A, Uldbjerg N, editors. Føtalmedicinsk og gynækologisk ultralydskanning. 2008.

(5) Evans MI, Kilpatrick M. Noninvasive prenatal diagnosis: 2010. Clin Lab Med 2010; 30(3):655-665.

(6) Jacobson CB, Barter RH. Intrauterine diagnosis and management of genetic defects. Am J Obstet Gynecol 1967; 99(6):796-807.

(7) Bang J, Northeved A. A new ultrasonic method for transabdominal amniocentesis. Am J Obstet Gynecol 1972; 114(5):599-601.

(8) Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. Lancet 1986; 1(8493):1287-1293.

(9) Sundberg K, Bang J, Smidt-Jensen S, Brocks V, Lundsteen C, Parner J et al. Randomised study of risk of fetal loss related to early amniocentesis versus chorionic villus sampling. Lancet 1997; 350(9079):697-703.

(10) DSOG Guideline CVS og amniocentese 2009
http://www.dsog.dk/sandbjerg/foeto/AC%20og%20CVS%202009%20forslag%2014%201%202009.pdf.
Ref Type: Internet Communication

(11) Smidt-Jensen S, Hahnemann N. Transabdominal fine needle biopsy from chorionic villi in the first trimester. Prenat Diagn 1984; 4(3):163-169.

(12) Froster UG, Baird PA. Limb-reduction defects and chorionic villus sampling. Lancet 1992; 339(8784):66.

(13) Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. Cochrane Database Syst Rev 2003;(3):CD003252.

(14) Smidt-Jensen S, Permin M, Philip J, Lundsteen C, Zachary JM, Fowler SE et al. Randomised comparison of amniocentesis and transabdominal and transcervical chorionic villus sampling. Lancet 1992; 340(8830):1237-1244.

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

(16) de G, I, Tijmstra T, Bleker OP, van Lith JM. Womens' preference in Down syndrome screening. Prenat Diagn 2002; 22(7):624-629.

(17) Ledbetter DH, Zachary JM, Simpson JL, Golbus MS, Pergament E, Jackson L et al. Cytogenetic results from the U.S. Collaborative Study on CVS. Prenat Diagn 1992; 12(5):317-345.

(18) Boormans EM, Birnie E, Oepkes D, Galjaard RJ, Schuring-Blom GH, van Lith JM. Comparison of multiplex ligation-dependent probe amplification and karyotyping in prenatal diagnosis. Obstet Gynecol 2010; 115(2 Pt 1):297-303.

(19) Faas BH, Cirigliano V, Bui TH. Rapid methods for targeted prenatal diagnosis of common chromosome aneuploidies. Semin Fetal Neonatal Med 2011; 16(2):81-87.

(20) Wald N, Cuckle H. Reporting the assessment of screening and diagnostic tests. Br J Obstet Gynaecol 1989; 96(4):389-396.

(21) Grimes DA, Schulz KF. Uses and abuses of screening tests. Lancet 2002; 359(9309):881-884.

(22) Morris JK, Wald NJ. The effect of correlations between screening markers on screening performance. J Med Screen 2007; 14(3):151-157.

(23) Wald N, Leck I. Tests using a single marker. Antenatal and neonatal screening. Second edition ed. Oxford 2000, University Press; 2000.

(24) Wald N, Leck I. Tests using multiples markers. In: Oxford University press 2000, editor. Antenatal and neonatal screening. Second edition ed. 2000.

(25) Penrose LS. The relative effects of paternal and maternal age in mongolism. 1933. J Genet 2009; 88(1):9-14.

(26) Snijders RJ, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age- and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol 1999; 13(3):167-170.

(27) Canick J. Prenatal screening for trisomy 21: recent advances and guidelines. Clin Chem Lab Med 2011.

(28) Sundhedsstyrelsen: Vejledning om prænatal genetisk information, rådgivning og undersøgelse (1994).
Ref Type: Report

(29) Report from working group 2003 *http://www.sst.dk/publ/Publ2003/fosterdiagnostik.pdf.*
Ref Type: Internet Communication

(30) http://www.statistikbanken.dk/.
Ref Type: Internet Communication

(31) Wald NJ, Cuckle HS, Densem JW, Nanchahal K, Royston P, Chard T et al. Maternal serum screening for Down's syndrome in early pregnancy. BMJ 1988; 297(6653):883-887.

(32) Christiansen M, Petersen PL, Permin M, Larsen LA, Norgaard-Pedersen B. [Maternal serum screening for congenital abnormalities and Down syndrome in Sonderjylland County. Eight years of experience]. Ugeskr Laeger 1999; 161(50):6928-6934.

(33) Norgaard-Pedersen B, Christiansen M. [Maternal serum screening for Down syndrome]. Ugeskr Laeger 1997; 159(41):6087-6088.

(34) Spencer K, Macri JN, Aitken DA, Connor JM. Free beta-hCG as first-trimester marker for fetal trisomy. Lancet 1992; 339(8807):1480.

(35) Wald N, Stone R, Cuckle HS, Grudzinskas JG, Barkai G, Brambati B et al. First trimester concentrations of pregnancy associated plasma protein A and placental protein 14 in Down's syndrome. BMJ 1992; 305(6844):28.

(36) Nicolaides KH, Azar G, Snijders RJ, Gosden CM. Fetal nuchal oedema: associated malformations and chromosomal defects. Fetal Diagn Ther 1992; 7(2):123-131.

(37) Azar GB, Snijders RJ, Gosden C, Nicolaides KH. Fetal nuchal cystic hygromata: associated malformations and chromosomal defects. Fetal Diagn Ther 1991; 6(1-2):46-57.

(38) Benacerraf BR, Gelman R, Frigoletto FD, Jr. Sonographic identification of second-trimester fetuses with Down's syndrome. N Engl J Med 1987; 317(22):1371-1376.

(39) Chervenak FA, Isaacson G, Blakemore KJ, Breg WR, Hobbins JC, Berkowitz RL et al. Fetal cystic hygroma. Cause and natural history. N Engl J Med 1983; 309(14):822-825.

(40) Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. BMJ 1992; 304(6831):867-869.

(41) 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; 7(4):245-250.

(42) Bilardo CM, Pajkrt E, de G, I, Mol BW, Bleker OP. Outcome of fetuses with enlarged nuchal translucency and normal karyotype. Ultrasound Obstet Gynecol 1998; 11(6):401-406.

(43) Bilardo CM. Increased nuchal translucency and normal karyotype: coping with uncertainty. Ultrasound Obstet Gynecol 2001; 17(2):99-101.

(44) Bilardo CM, Muller MA, Pajkrt E, Clur SA, van Zalen MM, Bijlsma EK. Increased nuchal translucency thickness and normal karyotype: time for parental reassurance. Ultrasound Obstet Gynecol 2007; 30(1):11-18.

(45) Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. Am J Obstet Gynecol 2005; 192(4):1005-1021.

(46) Bilardo CM, Timmerman E, Pajkrt E, van MM. Increased nuchal translucency in euploid fetuses--what should we be telling the parents? Prenat Diagn 2010; 30(2):93-102.

(47) Miltoft CB, Ekelund CK, Hansen BM, Lando A, Tabor A, Petersen OB et al. Increased nuchal translucency, normal karyotype and infant development. Ultrasound Obstet Gynecol 2011.

(48) Senat MV, Bussieres L, Couderc S, Roume J, Rozenberg P, Bouyer J et al. Long-term outcome of children born after a first-trimester measurement of nuchal translucency at the 99th percentile or greater with normal karyotype: a prospective study. Am J Obstet Gynecol 2007; 196(1):53-56.

(49) Hyett J, Moscoso G, Nicolaides K. Abnormalities of the heart and great arteries in first trimester chromosomally abnormal fetuses. Am J Med Genet 1997; 69(2):207-216.

(50) Hyett J, Perdu M, Sharland G, Snijders R, Nicolaides KH. Using fetal nuchal translucency to screen for major congenital cardiac defects at 10-14 weeks of gestation: population based cohort study. BMJ 1999; 318(7176):81-85.

(51) Hyett JA, Perdu M, Sharland GK, Snijders RS, Nicolaides KH. Increased nuchal translucency at 10-14 weeks of gestation as a marker for major cardiac defects. Ultrasound Obstet Gynecol 1997; 10(4):242-246.

(52) Daskalakis G, Sebire NJ, Jurkovic D, Snijders RJ, Nicolaides KH. Body stalk anomaly at 10-14 weeks of gestation. Ultrasound Obstet Gynecol 1997; 10(6):416-418.

(53) Sebire NJ, Snijders RJ, Davenport M, Greenough A, Nicolaides KH. Fetal nuchal translucency thickness at 10-14 weeks' gestation and congenital diaphragmatic hernia. Obstet Gynecol 1997; 90(6):943-946.

(54) Von Kaisenberg CS, Brand-Saberi B, Christ B, Vallian S, Farzaneh F, Nicolaides KH. Collagen type VI gene expression in the skin of trisomy 21 fetuses. Obstet Gynecol 1998; 91(3):319-323.

(55) Von Kaisenberg CS, Krenn V, Ludwig M, Nicolaides KH, Brand-Saberi B. Morphological classification of nuchal skin in human fetuses with trisomy 21, 18, and 13 at 12-18 weeks and in a trisomy 16 mouse. Anat Embryol (Berl) 1998; 197(2):105-124.

(56) Souka AP, Krampl E, Geerts L, Nicolaides KH. Congenital lymphedema presenting with increased nuchal translucency at 13 weeks of gestation. Prenat Diagn 2002; 22(2):91-92.

(57) Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004; 191(1):45-67.

(58) Pandya PP, Snijders RJ, Johnson SP, De Lourdes BM, Nicolaides KH. Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10 to 14 weeks of gestation. Br J Obstet Gynaecol 1995; 102(12):957-962.

(59) Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. Lancet 1998; 352(9125):343-346.

(60) Wojdemann KR, Shalmi AC, Christiansen M, Larsen SO, Sundberg K, Brocks V et al. Improved first-trimester Down syndrome screening performance by lowering the false-positive rate: a prospective study of 9941 low-risk women. Ultrasound Obstet Gynecol 2005; 25(3):227-233.

(61) Nicolaides KH, Snijders RJ, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. Prenat Diagn 1998; 18(5):519-523.

(62) Spencer K, Bindra R, Nix AB, Heath V, Nicolaides KH. Delta-NT or NT MoM: which is the most appropriate method for calculating accurate patient-specific risks for trisomy 21 in the first trimester? Ultrasound Obstet Gynecol 2003; 22(2):142-148.

(63) Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. A mixture model of nuchal translucency thickness in screening for chromosomal defects. Ultrasound Obstet Gynecol 2008; 31(4):376-383.

(64) Sahota DS, Leung TY, Chen M, Chan LW, Fung TY, Lau TK. Comparison of likelihood ratios of first-trimester nuchal translucency measurements: multiples of median, delta or mixture. Ultrasound Obstet Gynecol 2010; 36(1):15-19.

(65) Bonno M, Oxvig C, Kephart GM, Wagner JM, Kristensen T, Sottrup-Jensen L et al. Localization of pregnancy-associated plasma protein-A and colocalization of pregnancy-associated plasma protein-A messenger ribonucleic acid and eosinophil granule major basic protein messenger ribonucleic acid in placenta. Lab Invest 1994; 71(4):560-566.

(66) Kirkegaard I, Uldbjerg N, Oxvig C. Biology of pregnancy-associated plasma protein-A in relation to prenatal diagnostics: an overview. Acta Obstet Gynecol Scand 2010; 89(9):1118-1125.

(67) Bischof P, DuBerg S, Herrmann W, Sizonenko PC. Pregnancy-associated plasma protein-A (PAPP-A) and hCG in early pregnancy. Br J Obstet Gynaecol 1981; 88(10):973-975.

(68) Cole LA. New discoveries on the biology and detection of human chorionic gonadotropin. Reprod Biol Endocrinol 2009; 7:8.

(69) Herr F, Baal N, Reisinger K, Lorenz A, McKinnon T, Preissner KT et al. HCG in the regulation of placental angiogenesis. Results of an in vitro study. Placenta 2007; 28 Suppl A:S85-S93.

(70) Zygmunt M, Herr F, Keller-Schoenwetter S, Kunzi-Rapp K, Munstedt K, Rao CV et al. Characterization of human chorionic gonadotropin as a novel angiogenic factor. J Clin Endocrinol Metab 2002; 87(11):5290-5296.

(71) Spencer K. Aneuploidy screening in the first trimester. Am J Med Genet C Semin Med Genet 2007; 145C(1):18-32.

(72) Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. Ultrasound Obstet Gynecol 2008; 31(5):493-502.

(73) Sorensen T, Larsen SO, Christiansen M. Weight adjustment of serum markers in early first-trimester prenatal screening for Down syndrome. Prenat Diagn 2005; 25(6):484-488.

(74) Spencer K, Bindra R, Nicolaides KH. Maternal weight correction of maternal serum PAPP-A and free beta-hCG MoM when screening for trisomy 21 in the first trimester of pregnancy. Prenat Diagn 2003; 23(10):851-855.

(75) Madsen HN, Ball S, Wright D, Torring N, Petersen OB, Nicolaides KH et al. A reassessment of biochemical marker distributions in trisomy 21-affected and unaffected twin pregnancies in the first trimester. Ultrasound Obstet Gynecol 2011; 37(1):38-47.

(76) Spencer K, Kagan KO, Nicolaides KH. Screening for trisomy 21 in twin pregnancies in the first trimester: an update of the impact of chorionicity on maternal serum markers. Prenat Diagn 2008; 28(1):49-52.

(77) Miron P, Cote YP, Lambert J. Effect of maternal smoking on prenatal screening for Down syndrome and trisomy 18 in the first trimester of pregnancy. Prenat Diagn 2008; 28(3):180-185.

(78) Spencer K, Bindra R, Cacho AM, Nicolaides KH. The impact of correcting for smoking status when screening for chromosomal anomalies using maternal serum biochemistry and fetal nuchal translucency thickness in the first trimester of pregnancy. Prenat Diagn 2004; 24(3):169-173.

(79) Spencer K, Heath V, El-Sheikhah A, Ong CY, Nicolaides KH. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first trimester: a study of Oriental, Asian and Afro-Caribbean populations. Prenat Diagn 2005; 25(5):365-369.

(80) 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; 33(1):8-17.

(81) Madsen HN, Ekelund CK, Torring N, Ovesen PG, Friis-Hansen L, Ringholm L et al. Impact of type 1 diabetes and glycemic control on fetal aneuploidy biochemical markers. Acta Obstet Gynecol Scand 2011.

(82) Cowans NJ, Stamatopoulou A, Maiz N, Spencer K, Nicolaides KH. The impact of fetal gender on first trimester nuchal translucency and maternal serum free beta-hCG and PAPP-A MoM in normal and trisomy 21 pregnancies. Prenat Diagn 2009; 29(6):578-581.

(83) Brizot ML, Snijders RJ, Bersinger NA, Kuhn P, Nicolaides KH. Maternal serum pregnancy-associated plasma protein A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. Obstet Gynecol 1994; 84(6):918-922.

(84) Brizot ML, Snijders RJ, Butler J, Bersinger NA, Nicolaides KH. Maternal serum hCG and fetal nuchal translucency thickness for the prediction of fetal trisomies in the first trimester of pregnancy. Br J Obstet Gynaecol 1995; 102(2):127-132.

(85) Cuckle HS, van Lith JM. Appropriate biochemical parameters in first-trimester screening for Down syndrome. Prenat Diagn 1999; 19(6):505-512.

(86) Spencer K. Aneuploidy screening in the first trimester. Am J Med Genet C Semin Med Genet 2007; 145C(1):18-32.

(87) Wright D, Spencer K, Kagan KK, Torring N, Petersen OB, Christou A et al. First-trimester combined screening for trisomy 21 at 7-14 weeks' gestation. Ultrasound Obstet Gynecol 2010; 36(4):404-411.

(88) Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 1999; 13(4):231-237.

(89) Campbell S, Warsof SL, Little D, Cooper DJ. Routine ultrasound screening for the prediction of gestational age. Obstet Gynecol 1985; 65(5):613-620.

(90) Nguyen TH, Larsen T, Engholm G, Moller H. Evaluation of ultrasound-estimated date of delivery in 17,450 spontaneous singleton births: do we need to modify Naegele's rule? Ultrasound Obstet Gynecol 1999; 14(1):23-28.

(91) Olesen AW, Thomsen SG. Prediction of delivery date by sonography in the first and second trimesters. Ultrasound Obstet Gynecol 2006; 28(3):292-297.

(92) Taipale P, Hiilesmaa V. Predicting delivery date by ultrasound and last menstrual period in early gestation. Obstet Gynecol 2001; 97(2):189-194.

(93) Salomon LJ, Bernard M, Amarsy R, Bernard JP, Ville Y. The impact of crown-rump length measurement error on combined Down syndrome screening: a simulation study. Ultrasound Obstet Gynecol 2009; 33(5):506-511.

(94) van Heesch PN, Struijk PC, Laudy JA, Steegers EA, Wildschut HI. Estimating the effect of gestational age on test performance of combined first-trimester screening for Down syndrome: a preliminary study. J Perinat Med 2010; 38(3):305-309.

(95) De BP, Siccardi M, Volpe G, Famularo L, Santi F, Canini S. First-trimester screening for Down syndrome using nuchal translucency measurement with free beta-hCG and PAPP-A between 10 and 13 weeks of pregnancy--the combined test. Prenat Diagn 1999; 19(4):360-363.

(96) de G, I, 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; 19(5):458-462.

(97) Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. Prenat Diagn 1997; 17(9):821-829.

(98) 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; 25(3):221-226.

(99) Kagan KO, Etchegaray A, Zhou Y, Wright D, Nicolaides KH. Prospective validation of first-trimester combined screening for trisomy 21. Ultrasound Obstet Gynecol 2009; 34(1):14-18.

(100) Calda P, Sipek A, Gregor V. Gradual implementation of first trimester screening in a population with a prior screening strategy: population based cohort study. Acta Obstet Gynecol Scand 2010; 89(8):1029-1033.

(101) O'Leary P, Breheny N, Dickinson JE, Bower C, Goldblatt J, Hewitt B et al. First-trimester combined screening for Down syndrome and other fetal anomalies. Obstet Gynecol 2006; 107(4):869-876.

(102) Schielen PC, van Leeuwen-Spruijt M, Belmouden I, Elvers LH, Jonker M, Loeber JG. Multi-centre first-trimester screening for Down syndrome in the Netherlands in routine clinical practice. Prenat Diagn 2006; 26(8):711-718.

(103) Soergel P, Pruggmayer M, Schwerdtfeger R, Muhlhaus K, Scharf A. Screening for trisomy 21 with maternal age, fetal nuchal translucency and maternal serum biochemistry at 11-14 weeks: a regional experience from Germany. Fetal Diagn Ther 2006; 21(3):264-268.

(104) Hadlow NC, Hewitt BG, Dickinson JE, Jacoby P, Bower C. Community-based screening for Down's Syndrome in the first trimester using ultrasound and maternal serum biochemistry. BJOG 2005; 112(11):1561-1564.

(105) Nicolaides K. The 11-13+6 weeks scan. Fetal Medicine Foundation; 2004.

(106) Robinson HP, Fleming JE. A critical evaluation of sonar "crown-rump length" measurements. Br J Obstet Gynaecol 1975; 82(9):702-710.

(107) Green JJ, Hobbins JC. Abdominal ultrasound examination of the first-trimester fetus. Am J Obstet Gynecol 1988; 159(1):165-175.

(108) van Zalen-Sprock RM, Vugt JM, van Geijn HP. First-trimester sonography of physiological midgut herniation and early diagnosis of omphalocele. Prenat Diagn 1997; 17(6):511-518.

(109) Whitlow BJ, Economides DL. The optimal gestational age to examine fetal anatomy and measure nuchal translucency in the first trimester. Ultrasound Obstet Gynecol 1998; 11(4):258-261.

(110) Mulvey S, Baker L, Edwards A, Oldham J, Shekleton P, Wallace EM. Optimising the timing for nuchal translucency measurement. Prenat Diagn 2002; 22(9):775-777.

(111) 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; 20(3):219-225.

(112) Borrell A, Robinson JN, Santolaya-Forgas J. Report on the 11- to 13+6-week ultrasound evaluation as a screening test for trisomy 21 in singleton pregnancies. Am J Perinatol 2009; 26(10):703-710.

(113) Kirkegaard I, Petersen OB, Uldbjerg N, Torring N. Improved performance of first-trimester combined screening for trisomy 21 with the double test taken before a gestational age of 10 weeks. Prenat Diagn 2008; 28(9):839-844.

(114) Spencer K, Cuckle HS. Screening for chromosomal anomalies in the first trimester: does repeat maternal serum screening improve detection rates? Prenat Diagn 2002; 22(10):903-906.

(115) Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. N Engl J Med 1999; 341(7):461-467.

(116) Wald NJ, Rodeck C, Hackshaw AK, Rudnicka A. SURUSS in perspective. Semin Perinatol 2005; 29(4):225-235.

(117) Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R et al. First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med 2005; 353(19):2001-2011.

(118) Chervenak FA, McCullough LB. Ethical considerations in first-trimester Down syndrome risk assessment. Curr Opin Obstet Gynecol 2010; 22(2):135-138.

(119) Benn P, Wright D, Cuckle H. Practical strategies in contingent sequential screening for Down syndrome. Prenat Diagn 2005; 25(8):645-652.

(120) Christiansen M, Olesen LS. An increase in cost-effectiveness of first trimester maternal screening programmes for fetal chromosome anomalies is obtained by contingent testing. Prenat Diagn 2002; 22(6):482-486.

(121) Cuckle HS. Growing complexity in the choice of Down's syndrome screening policy. Ultrasound Obstet Gynecol 2002; 19(4):323-326.

(122) Kagan KO, Staboulidou I, Cruz J, Wright D, Nicolaides KH. Two-stage first-trimester screening for trisomy 21 by ultrasound assessment and biochemical testing. Ultrasound Obstet Gynecol 2010; 36(5):542-547.

(123) Sahota DS, Leung TY, Chan LW, Law LW, Fung TY, Chen M et al. Comparison of first-trimester contingent screening strategies for Down syndrome. Ultrasound Obstet Gynecol 2010; 35(3):286-291.

(124) Vadiveloo T, Crossley JA, Aitken DA. First-trimester contingent screening for Down syndrome can reduce the number of nuchal translucency measurements required. Prenat Diagn 2009; 29(1):79-82.

(125) Wald NJ, Rudnicka AR, Bestwick JP. Sequential and contingent prenatal screening for Down syndrome. Prenat Diagn 2006; 26(9):769-777.

(126) Wright D, Bradbury I, Benn P, Cuckle H, Ritchie K. Contingent screening for Down syndrome is an efficient alternative to non-disclosure sequential screening. Prenat Diagn 2004; 24(10):762-766.

(127) Wright D, Bradbury I, Cuckle H, Gardosi J, Tonks A, Standing S et al. Three-stage contingent screening for Down syndrome. Prenat Diagn 2006; 26(6):528-534.

(128) Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0 to 13+6 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33(1):18-22.

(129) Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33(5):512-517.

(130) Pandya PP, Hilbert F, Snijders RJ, Nicolaides KH. Nuchal translucency thickness and crown-rump length in twin pregnancies with chromosomally abnormal fetuses. J Ultrasound Med 1995; 14(8):565-568.

(131) Sebire NJ, Snijders RJ, Hughes K, Sepulveda W, Nicolaides KH. Screening for trisomy 21 in twin pregnancies by maternal age and fetal nuchal translucency thickness at 10-14 weeks of gestation. Br J Obstet Gynaecol 1996; 103(10):999-1003.

(132) Cuckle H, Maymon R. Down syndrome risk calculation for a twin fetus taking account of the nuchal translucency in the co-twin. Prenat Diagn 2010; 30(9):827-833.

(133) Wright D, Syngelaki A, Staboulidou I, Cruz JJ, Nicolaides KH. Screening for trisomies in dichorionic twins by measurement of fetal nuchal translucency thickness according to the mixture model. Prenat Diagn 2011; 31(1):16-21.

(134) Wojdemann KR, Larsen SO, Shalmi AC, Sundberg K, Tabor A, Christiansen M. Nuchal translucency measurements are highly correlated in both mono- and dichorionic twin pairs. Prenat Diagn 2006; 26(3):218-220.

(135) Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011; 31(1):7-15.

(136) Morris JK, Savva GM. The risk of fetal loss following a prenatal diagnosis of trisomy 13 or trisomy 18. Am J Med Genet A 2008; 146(7):827-832.

(137) Spencer K, Ong C, Skentou H, Liao AW, Nicolaides H. Screening for trisomy 13 by fetal nuchal translucency and maternal serum free beta-hCG and PAPP-A at 10-14 weeks of gestation. Prenat Diagn 2000; 20(5):411-416.

(138) Tul N, Spencer K, Noble P, Chan C, Nicolaides K. Screening for trisomy 18 by fetal nuchal translucency and maternal serum free beta-hCG and PAPP-A at 10-14 weeks of gestation. Prenat Diagn 1999; 19(11):1035-1042.

(139) Hyett JA, Noble PL, Snijders RJ, Montenegro N, Nicolaides KH. Fetal heart rate in trisomy 21 and other chromosomal abnormalities at 10-14 weeks of gestation. Ultrasound Obstet Gynecol 1996; 7(4):239-244.

(140) Liao AW, Snijders R, Geerts L, Spencer K, Nicolaides KH. Fetal heart rate in chromosomally abnormal fetuses. Ultrasound Obstet Gynecol 2000; 16(7):610-613.

(141) 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; 23(9):1968-1975.

(142) Spencer K, Nicolaides KH. A first trimester trisomy 13/trisomy 18 risk algorithm combining fetal nuchal translucency thickness, maternal serum free beta-hCG and PAPP-A. Prenat Diagn 2002; 22(10):877-879.

(143) Kagan KO, Wright D, Maiz N, Pandeva I, Nicolaides KH. Screening for trisomy 18 by maternal age, fetal nuchal translucency, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 2008; 32(4):488-492.

(144) Breathnach FM, Malone FD, Lambert-Messerlian G, Cuckle HS, Porter TF, Nyberg DA et al. First- and second-trimester screening: detection of aneuploidies other than Down syndrome. Obstet Gynecol 2007; 110(3):651-657.

(145) Bartlett LA, Berg CJ, Shulman HB, Zane SB, Green CA, Whitehead S et al. Risk factors for legal induced abortion-related mortality in the United States. Obstet Gynecol 2004; 103(4):729-737.

(146) Abele H, Wagner N, Hoopmann M, Grischke EM, Wallwiener D, Kagan KO. Effect of deviation from the mid-sagittal plane on the measurement of fetal nuchal translucency. Ultrasound Obstet Gynecol 2010; 35(5):525-529.

(147) Christiansen M, Larsen SO. Precision of risk in multidimensional likelihood ratio based screening with first trimester screening for Down's syndrome as a special case. J Med Screen 2001; 8(2):61-64.

(148) Kagan KO, Wright D, Etchegaray A, Zhou Y, Nicolaides KH. Effect of deviation of nuchal translucency measurements on the performance of screening for trisomy 21. Ultrasound Obstet Gynecol 2009; 33(6):657-664.

(149) Nix B, Wright D, Baker A. The impact of bias in MoM values on patient risk and screening performance for Down syndrome. Prenat Diagn 2007; 27(9):840-845.

(150) Wright D, Abele H, Baker A, Kagan KO. Impact of bias in serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A multiples of the median levels on first-trimester screening for trisomy 21. Ultrasound Obstet Gynecol 2011; 38(3):309-313.

(151) Sorensen S, Momsen G, Sundberg K, Friis-Hansen L, Jorgensen FS. First-trimester risk calculation for trisomy 13, 18, and 21: comparison of the screening efficiency between 2 locally developed programs and commercial software. Clin Chem 2011; 57(7):1023-1031.

(152) Herman A, Dreazen E, Maymon R, Tovbin Y, Bukovsky I, Weinraub Z. Implementation of nuchal translucency image-scoring method during ongoing audit. Ultrasound Obstet Gynecol 1999; 14(6):388-392.

(153) Koster MP, Wortelboer EJ, Engels MA, Stoutenbeek PH, Elvers LH, Visser GH et al. Quality of nuchal translucency measurements in The Netherlands: a quantitative analysis. Ultrasound Obstet Gynecol 2009; 34(2):136-141.

(154) Monni G, Zoppi MA, Ibba RM, Floris M. Fetal nuchal translucency test for Down's syndrome. Lancet 1997; 350(9091):1631-1632.

(155) Snijders RJ, Thom EA, Zachary JM, Platt LD, Greene N, Jackson LG et al. First-trimester trisomy screening: nuchal translucency measurement training and quality assurance to correct and unify technique. Ultrasound Obstet Gynecol 2002; 19(4):353-359.

(156) Wojdemann KR, Christiansen M, Sundberg K, Larsen SO, Shalmi A, Tabor A. Quality assessment in prospective nuchal translucency screening for Down syndrome. Ultrasound Obstet Gynecol 2001; 18(6):641-644.

(157) Pandya PP, Altman DG, Brizot ML, Pettersen H, Nicolaides KH. Repeatability of measurement of fetal nuchal translucency thickness. Ultrasound Obstet Gynecol 1995; 5(5):334-337.

(158) Pajkrt E, Mol BW, Boer K, Drogtrop AP, Bossuyt PM, Bilardo CM. Intra- and interoperator repeatability of the nuchal translucency measurement. Ultrasound Obstet Gynecol 2000; 15(4):297-301.

(159) Braithwaite JM, Kadir RA, Pepera TA, Morris RW, Thompson PJ, Economides DL. Nuchal translucency measurement: training of potential examiners. Ultrasound Obstet Gynecol 1996; 8(3):192-195.

(160) Abele H, Hoopmann M, Wright D, Hoffmann-Poell B, Huettelmaier M, Pintoffl K et al. Intra- and interoperator reliability of manual and semi-automated measurement of fetal nuchal translucency by sonographers with different levels of experience. Ultrasound Obstet Gynecol 2010; 36(4):417-422.

(161) Moratalla J, Pintoffl K, Minekawa R, Lachmann R, Wright D, Nicolaides KH. Semi-automated system for measurement of nuchal translucency thickness. Ultrasound Obstet Gynecol 2010; 36(4):412-416.

(162) Akolekar R, Perez Penco JM, Skyfta E, Rodriguez CJ, Nicolaides KH. Maternal serum placental protein 13 at eleven to thirteen weeks in chromosomally abnormal pregnancies. Fetal Diagn Ther 2010; 27(2):72-77.

(163) Bredaki FE, Wright D, Matos P, Syngelaki A, Nicolaides KH. First-Trimester Screening for Trisomy 21 Using Alpha-Fetoprotein. Fetal Diagn Ther 2011.

(164) Christiansen M, Oxvig C, Wagner JM, Qin QP, Nguyen TH, Overgaard MT et al. The proform of eosinophil major basic protein: a new maternal serum marker for Down syndrome. Prenat Diagn 1999; 19(10):905-910.

(165) Christiansen M, Norgaard-Pedersen B. Inhibin A is a maternal serum marker for Down's syndrome early in the first trimester. Clin Genet 2005; 68(1):35-39.

(166) Koster MP, Wortelboer EJ, Stoutenbeek P, Visser GH, Schielen PC. Modeling Down syndrome screening performance using first-trimester serum markers. Ultrasound Obstet Gynecol 2011; 38(2):134-139.

(167) Laigaard J, Sorensen T, Frohlich C, Pedersen BN, Christiansen M, Schiott K et al. ADAM12: a novel first-trimester maternal serum marker for Down syndrome. Prenat Diagn 2003; 23(13):1086-1091.

(168) Wald NJ, Watt HC, Norgaard-Pedersen B, Christiansen M. SP1 in pregnancies with Down syndrome in the first trimester of pregnancy. International Prenatal Screening Research Group. Prenat Diagn 1999; 19(6):517-520.

(169) Wright D, Spencer K, Nix B. First trimester screening for Down syndrome using free beta hCG, total hCG and PAPP-A: an exploratory study. Prenat Diagn 2007; 27(12):1118-1122.

(170) Christiansen M, Pihl K, Hedley PL, Gjerris AC, Lind PO, Larsen SO et al. ADAM 12 may be used to reduce the false positive rate of first trimester combined screening for Down syndrome. Prenat Diagn 2010; 30(2):110-114.

(171) Wright DE, Bradbury I. Repeated measures screening for Down's Syndrome. BJOG 2005; 112(1):80-83.

(172) Sonek J, Nicolaides K. Additional first-trimester ultrasound markers. Clin Lab Med 2010; 30(3):573-592.

(173) 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; 358(9294):1665-1667.

(174) Zoppi MA, Ibba RM, Axiana C, Floris M, Manca F, Monni G. Absence of fetal nasal bone and aneuploidies at first-trimester nuchal translucency screening in unselected pregnancies. Prenat Diagn 2003; 23(6):496-500.

(175) Orlandi F, Bilardo CM, Campogrande M, Krantz D, Hallahan T, Rossi C et al. Measurement of nasal bone length at 11-14 weeks of pregnancy and its potential role in Down syndrome risk assessment. Ultrasound Obstet Gynecol 2003; 22(1):36-39.

(176) Otano L, Aiello H, Igarzabal L, Matayoshi T, Gadow EC. Association between first trimester absence of fetal nasal bone on ultrasound and Down syndrome. Prenat Diagn 2002; 22(10):930-932.

(177) Sonek JD, Cicero S, Neiger R, Nicolaides KH. Nasal bone assessment in prenatal screening for trisomy 21. Am J Obstet Gynecol 2006; 195(5):1219-1230.

(178) Cicero S, Bindra R, Rembouskos G, Tripsanas C, Nicolaides KH. Fetal nasal bone length in chromosomally normal and abnormal fetuses at 11-14 weeks of gestation. J Matern Fetal Neonatal Med 2002; 11(6):400-402.

(179) Kagan KO, Cicero S, Staboulidou I, Wright D, Nicolaides KH. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33(3):259-264.

(180) Plasencia W, Dagklis T, Pachoumi C, Kolitsi E, Nicolaides KH. Frontomaxillary facial angle at 11 + 0 to 13 + 6 weeks: effect of plane of acquisition. Ultrasound Obstet Gynecol 2007; 29(6):660-665.

(181) Yagel S, Cohen SM, Messing B. First and early second trimester fetal heart screening. Curr Opin Obstet Gynecol 2007; 19(2):183-190.

(182) Kiserud T. Physiology of the fetal circulation. Semin Fetal Neonatal Med 2005; 10(6):493-503.

(183) Bilardo CM, Timmerman E, De Medina PG, Clur SA. Low-resistance hepatic artery flow in first-trimester fetuses: an ominous sign. Ultrasound Obstet Gynecol 2011; 37(4):438-443.

(184) Zvanca M, Gielchinsky Y, Abdeljawad F, Bilardo CM, Nicolaides KH. Hepatic artery Doppler in trisomy 21 and euploid fetuses at 11-13 weeks. Prenat Diagn 2011; 31(1):22-27.

(185) Borenstein M, Persico N, Kagan KO, Gazzoni A, Nicolaides KH. Frontomaxillary facial angle in screening for trisomy 21 at 11 + 0 to 13 + 6 weeks. Ultrasound Obstet Gynecol 2008; 32(1):5-11.

(186) Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005; 29(4):252-257.

(187) Nicolaides KH. Turning the pyramid of prenatal care. Fetal Diagn Ther 2011; 29(3):183-196.

(188) Pandya PP, Snijders RJ, Psara N, Hilbert L, Nicolaides KH. The prevalence of non-viable pregnancy at 10-13 weeks of gestation. Ultrasound Obstet Gynecol 1996; 7(3):170-173.

(189) Kramer MS, McLean FH, Boyd ME, Usher RH. The validity of gestational age estimation by menstrual dating in term, preterm, and postterm gestations. JAMA 1988; 260(22):3306-3308.

(190) Dias T, Arcangeli T, Bhide A, Napolitano R, Mahsud-Dornan S, Thilaganathan B. First-trimester ultrasound determination of chorionicity in twin pregnancy. Ultrasound Obstet Gynecol 2011.

(191) Sepulveda W, Sebire NJ, Hughes K, Odibo A, Nicolaides KH. The lambda sign at 10-14 weeks of gestation as a predictor of chorionicity in twin pregnancies. Ultrasound Obstet Gynecol 1996; 7(6):421-423.

(192) Oldenburg A, Rode L, Bodker B, Ersbak V, Holmskov A, Jorgensen FS et al. Influence of chorionicity on perinatal outcome in a large cohort of Danish twin pregnancies. Ultrasound Obstet Gynecol 2011.

(193) Sherer DM. Adverse perinatal outcome of twin pregnancies according to chorionicity: review of the literature. Am J Perinatol 2001; 18(1):23-37.

(194) Sperling L, Kiil C, Larsen LU, Qvist I, Schwartz M, Jorgensen C et al. Naturally conceived twins with monochorionic placentation have the highest risk of fetal loss. Ultrasound Obstet Gynecol 2006; 28(5):644-652.

(195) Sperling L, Tabor A. Twin pregnancy: the role of ultrasound in management. Acta Obstet Gynecol Scand 2001; 80(4):287-299.

(196) El KA, Nasr B, Nassar M, Bernard JP, Ville Y. First-trimester ultrasound examination and the outcome of monochorionic twin pregnancies. Prenat Diagn 2007; 27(10):922-925.

(197) Kagan KO, Gazzoni A, Sepulveda-Gonzalez G, Sotiriadis A, Nicolaides KH. Discordance in nuchal translucency thickness in the prediction of severe twin-to-twin transfusion syndrome. Ultrasound Obstet Gynecol 2007; 29(5):527-532.

(198) Sebire NJ, Souka A, Skentou H, Geerts L, Nicolaides KH. Early prediction of severe twin-to-twin transfusion syndrome. Hum Reprod 2000; 15(9):2008-2010.

(199) Clur SA, Ottenkamp J, Bilardo CM. The nuchal translucency and the fetal heart: a literature review. Prenat Diagn 2009; 29(8):739-748.

(200) Makrydimas G, Sotiriadis A, Ioannidis JP. Screening performance of first-trimester nuchal translucency for major cardiac defects: a meta-analysis. Am J Obstet Gynecol 2003; 189(5):1330-1335.

(201) Chelemen T, Syngelaki A, Maiz N, Allan L, Nicolaides KH. Contribution of ductus venosus Doppler in first-trimester screening for major cardiac defects. Fetal Diagn Ther 2011; 29(2):127-134.

(202) Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH. Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11-13 weeks. Prenat Diagn 2011; 31(1):90-102.

(203) Poon LC, Kametas NA, Maiz N, Akolekar R, Nicolaides KH. First-trimester prediction of hypertensive disorders in pregnancy. Hypertension 2009; 53(5):812-818.

(204) 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; 31(2):147-152.

(205) Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. Fetal Diagn Ther 2011; 29(2):148-154.

(206) http://etiskraad.dk/da-DK/Aktuelt/Hoeringssvar-og-udtalelser/2003/30-05-2003-fosterdiagnostik-og-risikovurdering.aspx. 2003.
Ref Type: Internet Communication

(207) Chasen ST, Skupski DW, McCullough LB, Chervenak FA. Prenatal informed consent for sonogram: the time for first-trimester nuchal translucency has come. J Ultrasound Med 2001; 20(11):1147-1152.

(208) Nicolaides KH, Chervenak FA, McCullough LB, Avgidou K, Papageorghiou A. Evidence-based obstetric ethics and informed decision-making by pregnant women about invasive diagnosis after first-trimester assessment of risk for trisomy 21. Am J Obstet Gynecol 2005; 193(2):322-326.

(209) http://www.eurocat-network.eu/content/Special-Report-Prenatal-Screening-Policies.pdf. 2010.
Ref Type: Internet Communication

(210) Solberg B. [Informed choice--important also for Norwegian pregnant women]. Tidsskr Nor Laegeforen 2009; 129(13):1346-1348.

(211) Muller MA, Bleker OP, Bonsel GJ, Bilardo CM. Women's opinions on the offer and use of nuchal translucency screening for Down syndrome. Prenat Diagn 2006; 26(2):105-111.

(212) Spencer K, Aitken D. Factors affecting women's preference for type of prenatal screening test for chromosomal anomalies. Ultrasound Obstet Gynecol 2004; 24(7):735-739.

(213) Muller MA, Bleker OP, Bonsel GJ, Bilardo CM. Nuchal translucency screening and anxiety levels in pregnancy and puerperium. Ultrasound Obstet Gynecol 2006; 27(4):357-361.

(214) Marteau TM, Dormandy E, Michie S. A measure of informed choice. Health Expect 2001; 4(2):99-108.

(215) van den BM, Timmermans DR, Ten Kate LP, van Vugt JM, van der WG. Are pregnant women making informed choices about prenatal screening? Genet Med 2005; 7(5):332-338.

(216) Dahl K, Hvidman L, Jorgensen FS, Henriques C, Olesen F, Kjaergaard H et al. First-trimester Down syndrome screening: pregnant women's knowledge. Ultrasound Obstet Gynecol 2011; 38(2):145-151.

(217) Dahl K, Hvidman L, Jorgensen FS, Kesmodel US. Knowledge of prenatal screening and psychological management of test decisions. Ultrasound Obstet Gynecol 2011; 38(2):152-157.

(218) Bekker HL, Hewison J, Thornton JG. Applying decision analysis to facilitate informed decision making about prenatal diagnosis for Down syndrome: a randomised controlled trial. Prenat Diagn 2004; 24(4):265-275.

(219) Garcia E, Timmermans DR, van LE. The impact of ethical beliefs on decisions about prenatal screening tests: searching for justification. Soc Sci Med 2008; 66(3):753-764.

(220) Zindler L. Ethical decision making in first trimester pregnancy screening. J Perinat Neonatal Nurs 2005; 19(2):122-131.

(221) www.DFMS.dk (The Society for Fetal Medicine in Denmark).
Ref Type: Internet Communication

(222) DSOG Biometriguideline
http://www.dsog.dk/sandbjerg/foeto/Biometriguideline%202008.pdf. 2008.
Ref Type: Internet Communication

(223) DSOG Nakkefoldsguideline
http://www.dsog.dk/sandbjerg/foeto/Nakkefoldsguidelineforslag_oktober09.pdf. 2009.
Ref Type: Internet Communication

Appendices (paper 1-4)

Paper 1

Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study. Ekelund CK, Jorgensen FS, Petersen OB, Sundberg K, Tabor A, BMJ 2008; 337:a2547

Paper 2

First trimester screening for Trisomy 21 in Denmark: Implications on detection and birth rates of Trisomy 18 and Trisomy 13. Ekelund CK, Petersen OB, Skibsted L, Kjaergaard S, Vogel I, Tabor A. Ultrasound Obstet Gynecol 2011;38(2):140-4

Paper 3

A prospective study evaluating the performance of first trimester combined screening for trisomy 21 using repeated sampling of the maternal serum markers PAPP-A and free β-hCG. Ekelund CK, Wright D, Ball S, Kirkegaard I, Nørgaard P, Sørensen S, Friis-Hansen L, Jørgensen FS, Uldbjerg N, Tørring N, Bech BH, Petersen OB, Tabor A. Submitted March 2012

Paper 4

Screening performance for trisomy 21 comparing first trimester combined screening and a first trimester contingent screening protocol including ductus venosus and tricuspid flow. Ekelund CK, Petersen OB, Sundberg K, Pedersen F, Vogel I, Tabor A. Submitted October 2011 to Prenatal diganosis, currently undergoing revision

BMJ

RESEARCH

Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study

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ABSTRACT

Objectives To evaluate the impact of a screening strategy in the first trimester, introduced in Denmark during 2004-6, on the number of infants born with Down's syndrome and the number of chorionic villus samplings and amniocenteses, and to determine detection and false positive rates in the screened population in 2005 and 2006.

Design Population based cohort study.

Setting 19 Danish departments of gynaecology and obstetrics and a central cytogenetic registry 2000-7.

Participants 65 000 pregnancies per year.

Main outcome measures The primary outcomes measured were number of fetuses and newborn infants with Down's syndrome diagnosed prenatally and postnatally and number of chorionic villus samplings and amniocenteses carried out. Secondary outcomes measured were number of women screened in 2005 and 2006, screen positive rate, and information on screening in 2005 and 2006 for infants with a postnatal diagnosis of Down's syndrome.

Results The number of infants born with Down's syndrome decreased from 55-65 per year during 2000-4 to 31 in 2005 and 32 in 2006. The total number of chorionic villus samplings and amniocenteses carried out decreased from 7524 in 2000 to 3510 in 2006. The detection rate in the screened population in 2005 was 86% (95% confidence interval 79% to 92%) and in 2006 was 93% (87% to 97%). The corresponding false positive rates were 3.9% (3.7% to 4.1%) and 3.3% (3.1% to 3.4%).

Conclusion The introduction of a combined risk assessment during the first trimester at a national level in Denmark halved the number of infants born with Down's syndrome. The strategy also resulted in a sharp decline in the number of chorionic villus samplings and amniocenteses carried out, even before full implementation of the policy.

INTRODUCTION

In September 2004 the Danish National Board of Health issued new guidelines for prenatal screening and diagnosis.[1] These recommended that pregnant women should be offered information about screening methods in pregnancy and, if desired, a combined risk assessment for Down's syndrome in the first trimester based on a combination of maternal age, nuchal translucency scanning, and a biochemical test for serum free β human chorionic gonadotrophin and pregnancy associated plasma protein A, called the double test. On the basis of this assessment women were to be informed about their risk (given as odds, such as 1:1250) of carrying a fetus with Down's syndrome. Women with a risk above a defined cut-off (for example, 1:300) were to be offered an invasive diagnostic procedure (chorionic villus sampling or amniocentesis). According to the previous guidelines from the Danish National Board of Health, pregnant women were to be offered chorionic villus sampling or amniocentesis if they were aged 35 or more, were at increased risk of carrying a fetus with Down's syndrome on the basis of serum screening using a triple test in the second trimester, or were at risk of an inherited disease. In 2000 the uptake of invasive diagnostic testing in women aged 35 or more was less than 50%, whereas around 20% of all pregnant women had nuchal translucency ultrasonography.[2] The triple test was not offered to all women but was done in about 10% of the population. Scans for malformations in the second trimester were offered to 28% of women.[2]

All 15 Danish counties decided to follow the guidelines from 2004 and introduce combined risk assessment in the first trimester. The cost of introducing the programme (ultrasound and laboratory equipment, training, wages for new staff) was covered by the counties and local hospitals. In 2004-6 the risk cut-off for referral to invasive diagnostic procedures varied between counties, from 1:250 to 1:400. The new policy was expected to detect 90% of fetuses with Down's syndrome at a 5% false positive rate on the basis of calculations made on the Danish population in 2001.

We evaluated the impact at a national level of the introduction of this new screening strategy on the number of infants born with Down's syndrome and on the number of chorionic villus samplings and amniocenteses. We also assessed whether the detection and false positive rates in the screened population for 2005 and 2006 were as expected.

METHODS

Denmark has a population of 5.4 million primarily white people and about 65 000 liveborn infants per

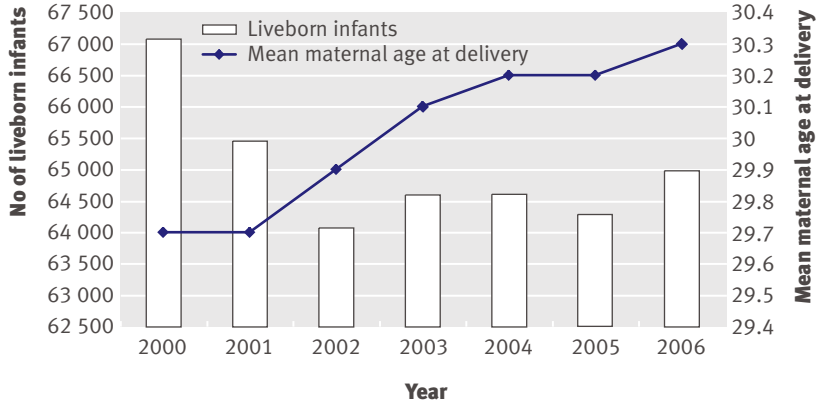

Fig 1 | Number of liveborn infants and mean maternal age at delivery in Denmark, 2000-6

year (www.statistikbanken.dk). At birth everyone is assigned a unique personal registration number, which is used for identification in the Danish social and healthcare system. This centralised, computer based, registration system enables follow-up of individuals through public registries.

From Statistics Denmark (www.statistikbanken.dk) we retrieved data on the number of liveborn infants born per year, the distribution of maternal age at delivery, and the mean maternal age at delivery for the period 2000-6. Using the maternal age specific risk of delivering an infant with Down's syndrome we calculated the expected number of liveborn infants with Down's syndrome.[3]

In Denmark results from prenatal and postnatal chromosome analyses are forwarded to the Danish central cytogenetic registry. From there we obtained information on the number of chorionic villus samplings and amniocenteses carried out during 2000-6, the indications for either procedure, and karyotypes.

In Denmark all newborn infants are examined by a midwife. When an abnormality or malformation such as Down's syndrome is suspected, follow-up with a paediatrician is initiated. The results of postnatal chromosome analysis including the personal registration numbers of the mother and infant are sent to the Danish central cytogenetic registry. The registry provided information on the number of infants with Down's syndrome born during 2000-4 as well as the personal registration number of all infants with Down's syndrome born during 2005-7 and their mothers.

For various political and practical reasons one county (Funen) had not yet reported the results of their chromosome analyses to the registry. We therefore obtained information separately on the number of chorionic villus samplings and amniocenteses and prenatal and postnatal cases of Down's syndrome for 2000-6 from Funen's chromosome laboratory.

Nuchal translucency ultrasonography is carried out by nurses, midwives, and doctors certified by and in accordance with the guidelines of the Fetal Medicine Foundation in London (www.fetalmedicine.com/). All obstetrics and gynaecology departments in Denmark use the same fetal medicine software program (Astraia, Germany) for calculating risk based on formulas derived by the Fetal Medicine Foundation. In some hospitals blood samples collected for the double test (serum free β human chorionic gonadotrophin and pregnancy associated plasma protein A) are analysed at local laboratories, whereas other hospitals send samples to a central laboratory. Most of the laboratories use Brahms Kryptor (Brahms, Immunodiagnostic Systems, UK) for biochemical analyses and a few use an alternative immunoassay (Delfia Xpress; PerkinElmer, Waltham, MA).

Evaluation of screening performance in 2005 and 2006

From the 19 obstetrics and gynaecology departments we collected information on the number of women who had had a risk assessment for Down's syndrome in the first trimester in 2005 and 2006, either as the optimal combined test (maternal age, nuchal translucency scan, and biochemistry) or by a combination of maternal age and nuchal translucency scan or biochemistry. To enable us to evaluate the screen positive rate, the departments reported the number of women given a risk assessment of 1:300 or more at the time of screening. We chose this uniform cut-off to simplify the presentation of data, despite some departments using a slightly different cut-off for referral to invasive diagnostic testing.

In the calculation of screening performance we included fetuses and newborn infants with Down's syndrome when a first trimester screening test had been done in 2005 or 2006. Information about gestational age at delivery for all infants with Down's syndrome born during 2005-7 was obtained from the Danish National Board of Health.

We cross checked the personal registration numbers of women who had given birth to an infant with Down's syndrome during 2005-7 with all Astraia database servers in Denmark to obtain information on whether screening had been carried out in the first trimester. Information about screening was also requested in those cases where Down's syndrome was diagnosed prenatally by an invasive procedure carried out for indications other than an increased risk of Down's syndrome.

RESULTS

A combined risk assessment in the first trimester was introduced successively in Denmark. In January 2005

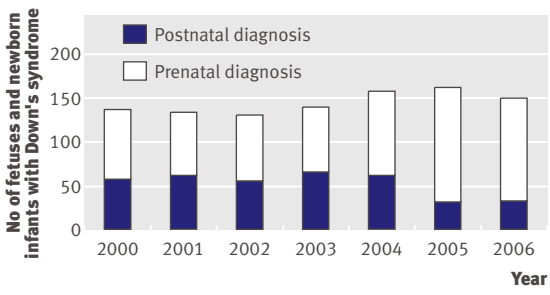

Fig 2 | Number of fetuses and newborn infants with Down's syndrome diagnosed prenatally or postnatally in Denmark, 2000-6

nine of the 15 counties (60%) offered women screening, increasing to 13 counties (87%) by January 2006. By June 2006 the whole of the country was covered.

The yearly number of deliveries in Denmark decreased slightly during 2000-6 (fig 1), whereas the mean maternal age at delivery increased from 29.7 years in 2000 to 30.3 years in 2006. Based on the actual distribution of maternal age and if no prenatal screening or invasive diagnosis had been carried out, the estimated expected number of infants with Down's syndrome increased from 121 in 2000 to 132 in 2005 and 135 in 2006.

Number of newborn infants with Down's syndrome

The number of newborn infants with Down's syndrome decreased from 55-65 per year in 2000-4 to 31 in 2005 and 32 in 2006. The total number of fetuses and newborn infants with Down's syndrome diagnosed prenatally or postnatally in 2000-3 was stable at around 135-140 per year, with an increase to 157 in 2004, 161 in 2005, and 149 in 2006 (fig 2). The proportion of cases diagnosed prenatally increased from 53-61% during 2000-4, to 81% in 2005 and 79% in 2006.

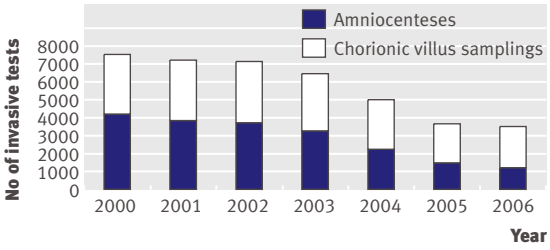

Fig 3 | Number of amniocenteses and chorionic villus samplings carried out in Denmark, 2000-6

Data on screening variables from 19 pregnancies in 2005 and 11 in 2006 resulting in newborn infants with Down's syndrome in Denmark

Year, maternal age* (years)	Nuchal translucency (mm)	Biochemistry performed	Risk assessment
2005:			
25	1.3	Yes	1:23641
26	2.4	Yes	1:793
28	1.5	Yes	1:2838
28	1.5	Yes	1:2598
29	1.7	No	1:4954
29	1.8	Yes	1:2980
30	1.9	Yes	1:1831
30	1.8	Yes	1:195†
32	1.8	Yes	1:627
32	1.8	Yes	1:3193
34	2.3	Yes	1:682
35	Reported as "normal"	No	1:1229
35	2.0	Yes	1:775
35	2.3	Yes	1:64†
36	1.9	Yes	1:3847
40	2.2	Yes	1:672
40	—	Yes	1:3‡
41	2.0	Yes	1:466
46	2.0	Yes	1:729
2006:			
24	1.6	Yes	1:1707
25	3.0	Yes	1:322
27	2.9	Yes	1:492
30	1.8	Yes	1:1764
31	1.7	Yes	1:79†
33	1.8	Yes	1:7693
34	1.5	Yes	1:3246
35	3.0	Yes	1:66†
37	6.3	Yes	1:5†
37	2.3	No (twins)	1:206†
40	2.3	Yes	1:79†

*Maternal age at time of nuchal translucency scan. If no scan was done then maternal age at week 12+4 is reported.
†Offered diagnostic testing.
‡Gestational age at screening 14+2, offered diagnostic testing.

Prenatal diagnostic procedure rate

The number of prenatal diagnostic procedures (chorionic villus samplings or amniocenteses) decreased from 7524 in 2000 to 3510 in 2006 (fig 3). The number of chorionic villus samplings decreased from 3322 in 2000 to 2302 in 2006, while the number of amniocenteses carried out decreased from 4202 to 1208 in the same years. This corresponds to an increase in the proportion of chorionic villus samplings from 44% to 66%.

Screening performance in 2005 and 2006

About 65 000 women were pregnant in Denmark during 2005-6. In 2005 40 815 women (62.8%) had a risk assessment carried out in the first trimester, increasing to 54 830 (84.4%) in 2006. The remaining women had no risk assessment done because they were offered an invasive diagnostic test for reasons other than a screen positive test result, declined screening, or failed to receive an offer for reasons such as residency in a county not yet offering screening. Figures 4 and 5 show the distribution of women eligible for screening and the groups in which infants with Down's syndrome were diagnosed prenatally and postnatally.

In 2005 a total of 1706 women (4.2%) had a risk of 1:300 or more (screen positive rate) and among these, 1388 women (81.4%) decided to have a diagnostic test (fig 4). Seventy two per cent of the diagnostic procedures done because of a screen positive risk assessment were chorionic villus samplings, the remainder were amniocenteses. In 2006 a total of 1899 women (3.5%) had a risk of 1:300 or more and 1704 (89.7%) underwent diagnostic testing as a consequence of the screening result. Seventy six per cent of the diagnostic procedures carried out because of a screen positive risk assessment were chorionic villus samplings.

In the population screened in 2005 the detection rate of Down's syndrome was 86% (95% confidence interval 79% to 92%)—(101+3)/(101+3+16+1)—as 104 of 121 women carrying a fetus with Down's syndrome were screened true positive (fig 4). Thus 17

women received a false negative screening result. One of these women had an amniocentesis on suspicion of a malformation after the 18-20 week scan, and the pregnancy was terminated (fig 4). An adjusted detection rate taking into account fetal loss from screening to time of birth (estimated as 25%[4]) was 82% (95% confidence interval 73% to 90%). The false positive rate was 3.9% (3.7% to 4.1%).

In 2006 the detection rate was 93% (87% to 97%)—(92+5)/(92+5+6+1)—as only seven women received a false negative screening result. One of these women had an amniocentesis on suspicion of a malformation, and the pregnancy was terminated (fig 5). The adjusted detection rate taking fetal loss into account was estimated at 92% (83% to 97%). The false positive rate was 3.3% (3.1% to 3.4%).

The odds of being affected (carrying a fetus with Down's syndrome) after receiving a screen positive risk assessment during the first trimester were 1:16 in 2005 and 1:20 in 2006. The odds of being affected after receiving a screen negative result were 1:2301 in 2005 and 1:7562 in 2006.

The odds of being affected after undergoing chorionic villus sampling or amniocentesis owing to advanced maternal age were similar in 2005 and 2006 (1:65 and 1:75); 15 fetuses with Down's syndrome were diagnosed among 980 women in 2005 and eight fetuses among 600 women in 2006. Indications other than advanced maternal age or high risk after screening for undergoing chorionic villus sampling or amniocentesis were mainly family history of chromosomal abnormality, mental retardation or monogenic inherited disease, or a high risk on the basis of serum screened in the second trimester.

Thirty infants with Down's syndrome were born to mothers who had had a risk assessment done in the first trimester during 2005 and 2006. The table gives the details of the risk assessments.

DISCUSSION

Even before full implementation of the policy for combined risk assessment during the first trimester in Denmark, the number of infants born with Down's syndrome decreased by about 50% and the number of cases diagnosed prenatally increased by around 30%.

The number of fetuses and newborn infants with Down's syndrome diagnosed prenatally or postnatally increased in the period 2000-5, with a slight decline in 2006 (fig 2). This was partly due to increasing maternal age, but was as expected because more fetuses with Down's syndrome are lost spontaneously than those that are chromosally normal. This increased rate has been estimated at around 25% from week 14 to term.[4] Based on the known distribution of maternal age at delivery in 2005 and 2006, 132 and 135 infants with Down's syndrome would have been expected in our population of 65 000 liveborn infants if the mothers had no prenatal intervention. Down's syndrome was diagnosed in 31 infants postnatally and 130 prenatally in 2005 and in 32 infants postnatally and 117 prenatally in 2006. Given a rate for fetal loss of 25%, this corresponds to 129 infants with Down's syndrome diagnosed postnatally in 2005 and 120 diagnosed postnatally in 2006. In 2005 the expected numbers

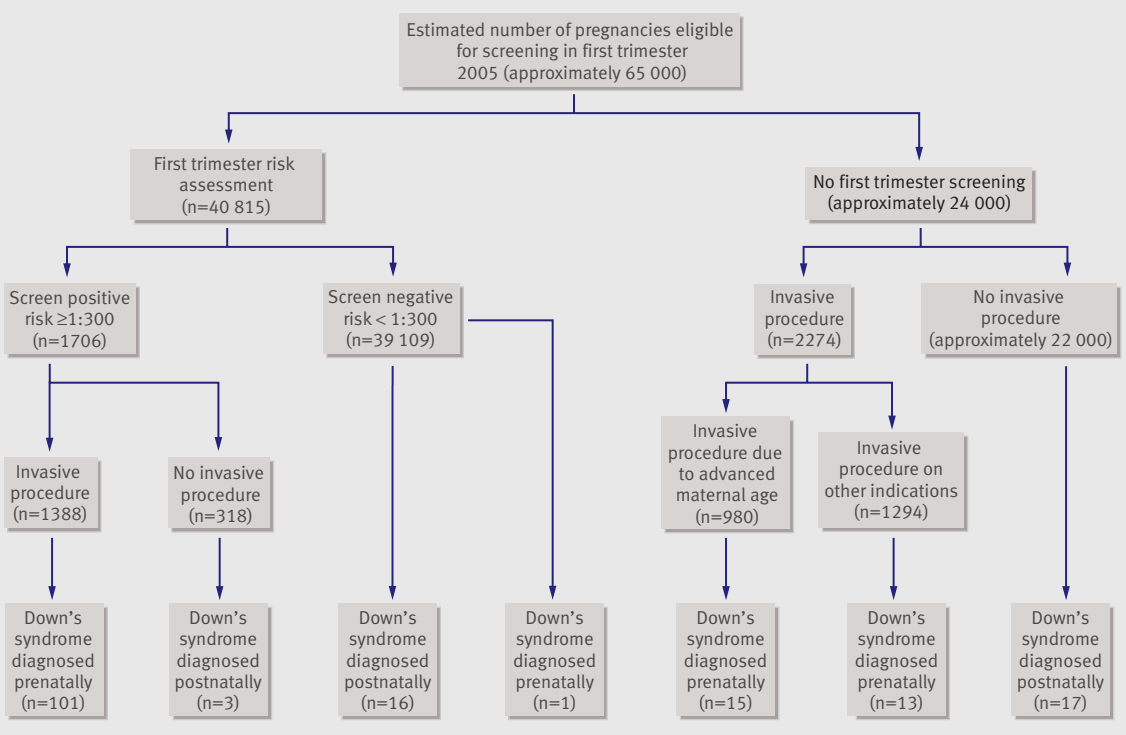

Fig 4 | Number of fetuses and newborn infants with Down's syndrome diagnosed prenatally or postnatally according to screening results in Denmark, 2005. Invasive procedures are chorionic villus samplings or amniocenteses

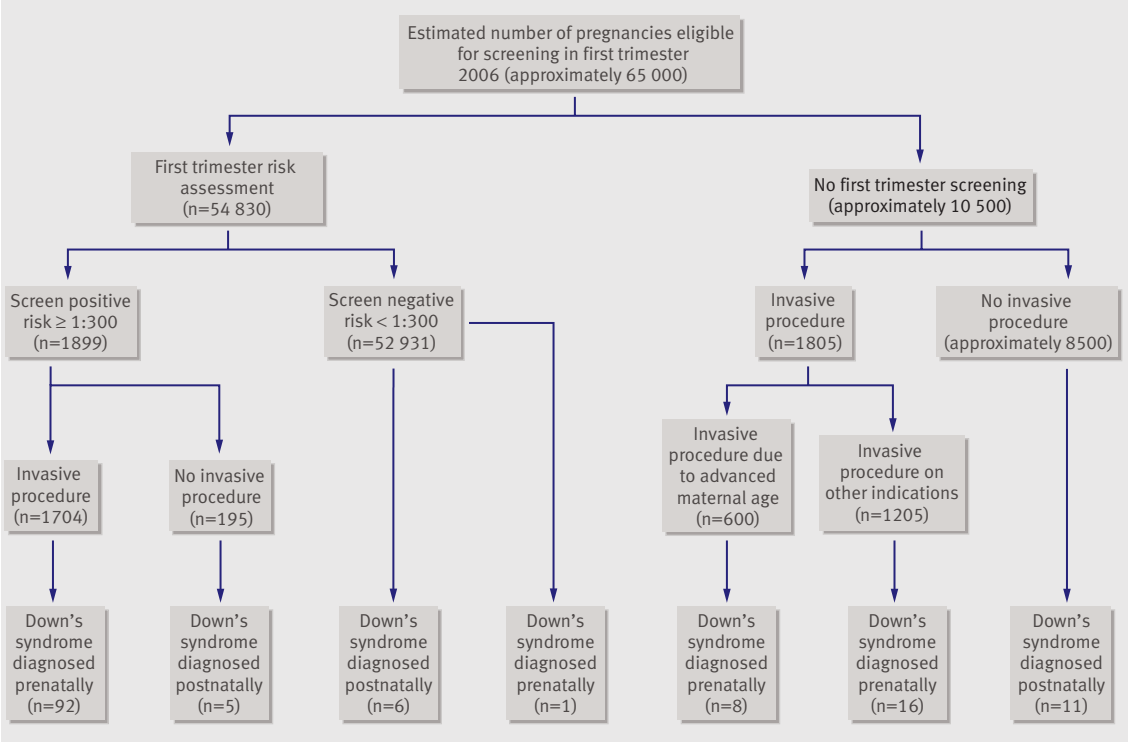

Fig 5 | **Number of fetuses and newborn infants with Down's syndrome diagnosed prenatally or postnatally according to screening results in Denmark, 2006. Invasive procedures are chorionic villus samplings or amniocenteses**

were close to those reported, whereas in 2006 the reported number was lower than expected. This may be due to chance fluctuation, as we believe follow-up is complete. The follow-up time for the numbers reported from 2006 is, however, relatively short and a few cases may therefore still be reported.

In 2005 national screening was not fully implemented. One third of the women were either not offered screening or declined. These women gave birth to a total of 17 infants with Down's syndrome. In 2006 the proportion of non-screened women decreased to 15%, as screening was then available for most women. About 8500 women who were not offered screening or declined screening or a diagnostic test in 2006 gave birth to a total of 11 infants with Down's syndrome. The national guidelines on prenatal screening emphasise that risk assessment for Down's syndrome should be done only if women choose the test on the basis of an informed choice. Therefore despite the programme now being accessible to all pregnant women in Denmark, it is expected that a proportion will still choose not to be screened. The size of this proportion when screening is fully available remains to be established; however, in 2005 only 2% of the population in two counties declined screening.[5] Studies on Danish women's attitude, knowledge about screening, and choice of test are ongoing.[6]

We found that the number of prenatal diagnostic tests (chorionic villus samplings and amniocenteses) carried out yearly decreased by more than 50% during 2000-6. A decrease in the number of prenatal diagnostic procedures could be seen even before the policy was changed, probably because pregnant women became aware of alternative prenatal investigations such as nuchal translucency scanning (fig 3). This was certainly the case in and around Copenhagen, when a prospective study of around 10 000 women was done in 1998-2001.[7] Nuchal translucency scanning was introduced in some departments even before the national guidelines were changed.

In 2005 and 2006 about 3% of women still had an invasive diagnostic procedure done because of indications other than a screen positive test result, with a tendency towards a reduced number of tests from 2005 to 2006 (2274 women in 2005, 1805 in 2006). The decrease was mainly due to fewer women choosing invasive diagnostic tests on the basis of advanced maternal age, as 980 invasive procedures were carried out for that indication in 2005 but decreased to 600 in 2006. The relatively high number of women choosing chorionic villus sampling or amniocentesis was probably partly due to lack of implementation of the new screening programme. It is also possible that women who had a diagnostic procedure for a previous pregnancy because they were aged 35 or more may have requested a diagnostic test again. When the new screening strategy based on ultrasound and biochemistry has been available for some years we expect the number of invasive diagnostic tests done because of advanced maternal age to decrease even further.

We found that 10-20% of women with a screen positive test result did not undergo an invasive diagnostic test. This is in accordance with reports from the Copenhagen First Trimester Study.[7] For

WHAT IS ALREADY KNOWN ON THIS TOPIC

Many countries are currently trying to achieve national screening strategies for Down's syndrome

None has described how a combined screening strategy in the first trimester affects numbers of infants born with Down's syndrome or rate of invasive procedures

Detection rates and false positive rates for the combined first trimester risk assessment have been reported only from specialised centres or from regional experience

WHAT THIS STUDY ADDS

After implementation of a national screening policy in Denmark, the number of infants born with Down's syndrome and the rate of invasive procedures was noticeably reduced

The screening strategy achieved high detection rates and low false positive rates

various reasons (advanced maternal age, conception by assisted reproduction technologies, or risk near the cut-off) some women do not want an invasive diagnostic test, probably because of the associated risk of miscarriage.

The difference in odds of carrying a fetus with Down's syndrome for those who were tested because of a screen positive risk assessment (1:16 in 2005, 1:20 in 2006) compared with that of being tested because of advanced maternal age (1:65 in 2005, 1:75 in 2006) clearly illustrates the rationale in screening using a combined risk assessment in the first trimester. As expected, this strategy reduces the number of unnecessary diagnostic procedures. The procedure related risk of miscarriage after chorionic villus sampling or amniocentesis is reported to be 1%.[8] In the group of women having an invasive diagnostic test done because of advanced maternal age in 2005 and 2006 16 chromosomally normal fetuses would then have been miscarried to diagnose 23 cases of Down's syndrome. This should be compared with the 31 fetuses possibly miscarried to diagnose 193 cases of Down's syndrome in the group of women with a screen positive test result. Combined risk assessment in the first trimester is not only a more effective screening method than maternal age alone, it also reduces the risk of miscarrying chromosomally normal fetuses when used as reason to be referred for testing instead of maternal age. Thus the false positive rate of prenatal diagnostic testing has been much reduced by changing the selection criterion from maternal age to risk assessment in the first trimester. The false negative rate has also changed: previously those women who chose to have chorionic villus sampling or amniocentesis because of advanced maternal age had a diagnostic test. Currently women choose to have a screening test; 0.4 women per 1000 in 2005 and 0.1 per 1000 in 2006 subsequently delivered a child with Down's syndrome, despite having a risk assessment below the 1:300 cut-off. These few women may feel more resentment towards the system that failed them than those women who chose not to have an invasive diagnostic test because of advanced maternal age. This emphasises the importance of informing all women about the limitations of screening.

For false positive rates of 3.9% and 3.3% in the screened populations we found detection rates for Down's syndrome of 86% in 2005 and 93% in 2006. This is in accordance with the screening performance expected by the Danish National Board of Health when it decided to implement this new screening strategy. This performance may be considered high, especially as the programme in 2005 and 2006 in many centres used a completely new screening method. Furthermore, we report the result of routine clinical practice, where not all risk assessments are based on the optimal variables (combination of maternal age, nuchal translucency scan, and biochemistry) as some are given only on maternal age and nuchal translucency scan or biochemistry. Other authors have also reported screening results achieved in routine clinical practice in up to 13 centres, with detection rates between 83% and 93% and false positive rates between 3.9% and 5.9%.[9-13] One study collected data from 44 centres in the Netherlands and found a detection rate of 71% for a false positive rate of 4.7%.[14] The authors explain the relatively low detection rate by too small measurements used for nuchal translucency, and expect to improve the detection rate by establishing quality assurance on the measurements. In a large prospective multicentre study the detection rate using a combined screening programme in the first trimester was 92.6% for a false positive rate of 5.2%.[15] Our data show that it is possible to introduce this screening strategy in as many as 19 different centres and still obtain national detection and false positive rates similar to those from specialised centres.

It is well known that implementation of new screening strategies requires effort, and many countries are currently facing various problems in trying to achieve a national strategy.[16-18] In Denmark, with its public, free of charge hospital system, we have succeeded in establishing a strong national organisation for fetal medicine. Recruitment and training of sonographers as well as quality control are in accordance with the guidelines from the Fetal Medicine Foundation in London (www.fetalmedicine.com). We have implemented national guidelines on screening in the first trimester, and from 1 January 2007 a common cut-off of 1:300 for referral to invasive diagnostic testing at the time of screening. Furthermore, the use of the same database software in all departments allows national data to be merged. A national quality database has been established that merges data from all fetal medicine units, the Danish national cytogenetic registry, and the national patient registry. This will allow follow-up of all screened women at a national level, as well as monitoring of detection rates, false positive rates, and invasive testing rates, a quality control that is considered essential after the implementation of a new screening programme.

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Contributors: AT and CKE planned and did the study in cooperation with FSJ, OBP, and KS. CKE and AT wrote the manuscript and are guarantors. Hans Jakob Andersen, Jeanette Christensen, Vibeke Ersbak, Richard Farlie, Carsten Henriques, Annette Wind Olesen, Anni Holmskov, Lisa Neerup Jensen, FSJ, Anette Kristiansen, Torben Larsen, OBP, Hedvig Poulsen, Jan Ramb, Lillian Skibsted, Peter Skovbo, Steffen Sommer, Lene Sperling, KS, Susanne Vemmelund Juul and Helle Zingenberg retrieved data from the local Astraia servers and reviewed and accepted the final manuscript.

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1 Danish National Board of Health. Report from a working commission "Prenatal diagnosis and risk assessment." www.sst.dk/upload/fosterdiagnostik1_001.pdf. 2003.
2 Jorgensen FS. [Organization of obstetric ultrasound in Denmark 2000. Description of the development since 1990]. *Ugeskr Laeger* 2003;165:4404-9.
3 Morris JK, Mutton DE, Alberman E. Revised estimates of the maternal age specific live birth prevalence of Down's syndrome. *J Med Screen* 2002;9:2-6.
4 Snijders RJ, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age- and gestation-specific risk for trisomy 21. *Ultrasound Obstet Gynecol* 1999;13:167-70.
5 Torring N, Jolving LR, Petersen OB, Holmskov A, Hertz JM, Uldbjerg N. [Prenatal diagnostics in Arhus and Viborg counties after implementation of first trimester risk assessment]. *Ugeskr Laeger* 2008;170:50-4.
6 Bangsgaard L, Tabor A. OC30: Are pregnant women and their partners making an informed choice about first-trimester risk assessment for Down syndrome? *Ultrasound Obstet Gynecol* 2007;30:376.
7 Wojdemann KR, Shalmi AC, Christiansen M, Larsen SO, Sundberg K, Brocks V, et al. Improved first-trimester Down syndrome screening performance by lowering the false-positive rate: a prospective study of 9941 low-risk women. *Ultrasound Obstet Gynecol* 2005;25:227-33.
8 Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev* 2003;(3):CD003252.
9 Hadlow NC, Hewitt BG, Dickinson JE, Jacoby P, Bower C. Community-based screening for Down's syndrome in the first trimester using ultrasound and maternal serum biochemistry. *Br J Obstet Gynaecol* 2005;112:1561-4.
10 O'Leary P, Breheny N, Dickinson JE, Bower C, Goldblatt J, Hewitt B, et al. First-trimester combined screening for Down syndrome and other fetal anomalies. *Obstet Gynecol* 2006;107:869-76.
11 Soergel P, Pruggmayer M, Schwerdtfeger R, Muhlhaus K, Scharf A. Screening for trisomy 21 with maternal age, fetal nuchal translucency and maternal serum biochemistry at 11-14 weeks: a regional experience from Germany. *Fetal Diagn Ther* 2006;21:264-8.
12 Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. 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.
13 Stenhouse EJ, Crossley JA, Aitken DA, Brogan K, Cameron AD, Connor JM. First-trimester combined ultrasound and biochemical screening for Down syndrome in routine clinical practice. *Prenat Diagn* 2004;24:774-80.
14 Schielen PC, van Leeuwen-Spruijt M, Belmouden I, Elvers LH, Jonker M, Loeber JG. Multi-centre first-trimester screening for Down syndrome in the Netherlands in routine clinical practice. *Prenat Diagn* 2006;26:711-8.
15 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;25:221-6.
16 Chang TC. Antenatal screening for Down syndrome in New Zealand: time for a national screening policy? *Aust N Z J Obstet Gynaecol* 2006;46:92-6.
17 O'Leary P, Breheny N, Reid G, Charles T, Emery J. Regional variations in prenatal screening across Australia: stepping towards a national policy framework. *Aust N Z J Obstet Gynaecol* 2006;46:427-32.
18 Reddy UM, Mennuti MT. Incorporating first-trimester Down syndrome studies into prenatal screening: executive summary of the National Institute of Child Health and Human Development workshop. *Obstet Gynecol* 2006;107:167-73.

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First-trimester screening for trisomy 21 in Denmark: implications for detection and birth rates of trisomy 18 and trisomy 13

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KEYWORDS: birth rates; Down syndrome; first-trimester screening; trisomy 13; trisomy 18

ABSTRACT

Objectives *In Denmark a new national guideline for prenatal screening and diagnosis was issued in 2004 according to which all pregnant women should be offered a first-trimester combined risk assessment for trisomy 21 (T21). The aim of this study was to investigate whether the new screening strategy for T21 has changed the gestational age at which trisomy 18 (T18) and trisomy 13 (T13) are diagnosed prenatally, and the number of infants born with T18 or T13.*

Methods *We collected from the Danish Cytogenetic Central Register information on all prenatal and postnatal chromosome analyses for T18 or T13, registered from 1997 to 2007. Information on first-trimester screening results was collected from each department of obstetrics and gynecology performing the nuchal translucency scans. The cut-off used for referral to invasive diagnostic testing for T21 and for T18/T13 was 1 : 300 and 1 : 150 at screening, respectively.*

Results *In total, there were 435 cases with T18 and 168 cases with T13 between 1997 and 2007 in Denmark. The estimated incidence of T18 and T13 at the time of delivery was calculated as 2.5 and 1.6 per 10 000 deliveries, respectively. The number (proportion) of cases diagnosed before week 18 increased significantly, from 63 (59.4%) in 1997 and 1998 to 90 (80.4%) in 2006 and 2007 (*$P < 0.001$*). In addition, the number of T18 and T13 cases diagnosed prenatally after week 22 or postnatally decreased significantly, from 34 (32.1%) in 1997 and 1998 to seven (6.3%) in 2006 and 2007 (*$P < 0.0001$*). For women participating in first-trimester risk assessment in 2006 and 2007, the detection rate of T18 and T13 was 78.8% (95% CI, 71.0–86.7%).*

Conclusion *The number of T18 and T13 fetuses diagnosed before week 18 increased significantly after the introduction of a combined first-trimester screening strategy for T21 in Denmark. In addition, the total number of fetuses diagnosed late in pregnancy and infants born with T18 or T13 decreased significantly. The national detection rate for T18 and T13 in the first trimester is comparable with detection rates found in modeled datasets and other prospective studies.*

INTRODUCTION

Trisomy 18 (T18) and trisomy 13 (T13) are, after trisomy 21 (T21), the most common autosomal trisomies observed in fetuses and newborns. They are associated with severe malformations and mental retardation and with a very high rate of intrauterine death[1]. The majority of infants born with T18 or T13 survive for only a few days[2–4].

It is well established that it is possible to screen for T21 in pregnancy using ultrasound and biochemical markers[5–10], and additional screening for T18 and T13 can be performed using the same markers[11,12]. These markers are maternal age, fetal nuchal translucency thickness, maternal-serum pregnancy-associated plasma protein-A (PAPP-A) and maternal-serum free beta-human chorionic gonadotropin (β-hCG). T21, T18 and T13 are

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all associated with increased maternal age, increased nuchal translucency and decreased PAPP-A. T18 and T13 are also associated with a decreased level of free β-hCG[7,11]. It has been estimated that up to 95% of fetuses with T18 or T13 can be detected using specific T18 and T13 risk algorithms in the first trimester[11].

Before 2004, Danish women were offered diagnostic testing (chorionic villus sampling (CVS) or amniocentesis (AC)) if they were considered to be at increased risk of carrying a fetus with T21 on the basis of maternal age (≥ 35 years of age), on serum screening results of the triple test in the second trimester or if the fetus was at risk of a diagnosable inherited disease. The new national guideline for Prenatal Screening and Diagnosis issued in 2004 by the National Board of Health recommends that all pregnant women should be offered information about screening methods in pregnancy, and if desired, a first-trimester combined risk assessment for T21. In 2006, 84% of the pregnant women in Denmark underwent first-trimester risk assessment, and this proportion increased to more than 90% in 2007[10,13].

The primary aim of this study was to investigate whether the introduction of the new screening strategy for T21 in Denmark has resulted in an earlier diagnosis of T18 and T13 in pregnancy, and in a reduction in the number of infants born with T18 or T13. In addition, we wanted to specifically evaluate the first-trimester combined screening performance for T18 and T13 in 2006 and 2007.

SUBJECTS AND METHODS

We collected information on the overall birth rate and mean maternal age at delivery from 1987 to 2007 through Statistic Denmark (www.statistikbanken.dk, June 2010). To be able to compare our observed incidence of T18 and T13 with those reported in previous publications where the incidence at time of delivery is reported, we calculated the estimated incidence at time of delivery using the observed number of cases, the gestational age at diagnosis and the estimated fetal loss rates given by Morris *et al.*[1]. To compare our observed incidence of T18 and T13 with the expected incidence in the Danish pregnant population we used the estimates of age-specific risks for T18 and T13 provided by Snijders *et al.*[14] and the Danish maternal age distribution for 1997–2007 provided by Statistic Denmark.

We collected information on all prenatal and postnatal chromosome analyses for T18 or T13, registered from 1997 to 2007, from the Danish Cytogenetic Central Register (DCCR). The DCCR receives a copy of all chromosome analysis results from the five cytogenetic laboratories in Denmark. Chromosome analyses include counting at least 10 metaphases, three of which are fully karyotyped. The prenatal cases with T18 or T13 were diagnosed on tissue from prenatal invasive diagnostic tests (CVS or AC) or from induced abortions. The few cases registered each year in relation to miscarriages were excluded, as chromosome analyses of miscarriages are only performed sporadically and do not reflect the actual number of cases. Cases detected with mosaicism were not included in the study population.

In Denmark, all persons have a unique and lifelong personal identification number, under which all reports to national registers are made. For each case we retrieved the maternal personal identification number and the indication for performing a chromosome analysis. The time of sampling was also retrieved: either the gestational age at which the sample was collected for karyotyping, in cases of prenatal testing; or the age of the infant, in cases of postnatal testing. In addition, we collected the date of death for the infants born with T18 or T13. Maternal age at sampling was calculated using maternal birth date and sampling date.

We divided all T18 and T13 cases into three groups: those diagnosed before gestational week 18; those diagnosed between gestational weeks 18 and 22; and those diagnosed after gestational week 22 or postnatally. The second-trimester anomaly scans in Denmark are performed in weeks 19–20. The 18-week cut-off defining early diagnosis was chosen because we anticipated that the first-trimester combined screening strategy in Denmark would increase the number of cases diagnosed before the anomaly scan.

To assess the first-trimester screening performance, we collected information, from the different hospitals' ultrasound databases (Astraia), on the screening results of mothers whose fetus or infant had T18 or T13 in 2006 and 2007. We used the unique personal identification number to merge the data from the DCCR and the Astraia databases. Following implementation of the national screening strategy, all obstetric departments in Denmark have offered first-trimester combined risk assessment since June 2006[10]. The cut-off for referral to invasive diagnostic testing (CVS or AC) for T21 and T18/T13, used in the majority of departments, is 1 : 300 and 1 : 150 at screening, respectively. The new guideline also recommended a second-trimester anomaly scan for all pregnant women. Prior to 2004, second-trimester anomaly scans were only offered in some regions in Denmark.

Differences between binary groups were compared using the chi-square test and $P < 0.05$ was considered significant.

The study was approved by the Data Protection Agency in Denmark (2010-41-4799).

RESULTS

During the study period (1997–2007) a total of 719 215 infants were liveborn in Denmark. Mean maternal age at delivery has increased during the last 20 years in Denmark, from 26.5 years in 1987 to 29.4 years in 1997 and 30.4 years in 2007. Figure 1 shows the number of infants born each year and mean maternal age at delivery in the period 1997 to 2007 in Denmark.

The estimated incidences of T18 and T13 livebirths in the Danish pregnant population in the time-period 1997–2007, given the known maternal age distribution and estimated risk provided by Snijders *et al.*[14], were 1.7

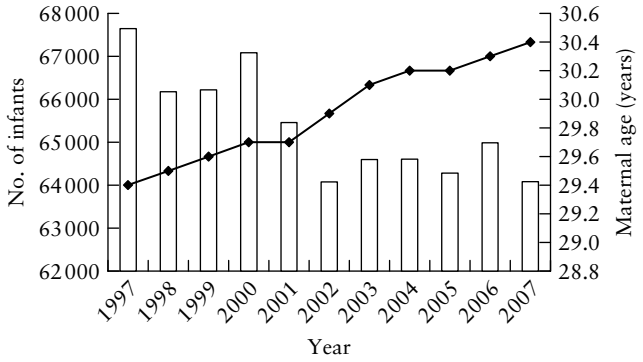

Figure 1 Number of infants born each year (□) and mean maternal age at delivery (—◆—) in Denmark from 1997 to 2007.

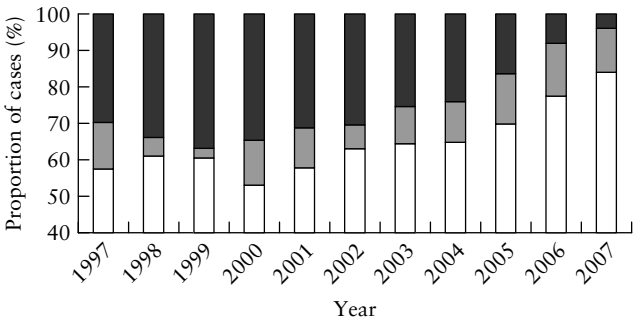

Figure 2 Proportion of cases with trisomy 18 or trisomy 13 according to age at diagnosis: after week 22 or postnatally (■); at gestational weeks 18–22 (▩); before gestational week 18 (□).

per 10 000 deliveries for T18 and 0.7 per 10 000 deliveries for T13.

There were 435 cases with T18 and 168 cases with T13 diagnosed prenatally or postnatally in the period 1997–2007. The estimated incidence of T18 at the time of delivery, using the number of observed cases and taking into account the estimated fetal loss rates reported by Morris *et al.*[1], was calculated as 2.5 per 10 000 deliveries. For T13 the estimated incidence was 1.6 per 10 000 deliveries.

The median maternal age at diagnosis of a fetus or infant with T18 or T13 was 35 (range, 17–48) years and 33 (range, 19–47) years, respectively.

The median survival for infants born with T18 was 5 (range, 0–554) days, and 30% survived for longer than 1 month. At the time of follow-up (December 2009), one child born in 2001 was still alive (diagnosed postnatally with T18 by chromosome analysis of blood). The median survival for infants born with T13 was 4.5 (range, 0–396) days, and 24% survived for longer than 1 month.

The number of T18 and T13 cases diagnosed prenatally after week 22, or postnatally, decreased significantly, from 34 in 1997 and 1998 to seven in 2006 and 2007 ($P < 0.0001$). In addition, the number of cases diagnosed before week 18 increased significantly, from 63 in 1997 and 1998 to 90 in 2006 and 2007 ($P < 0.001$). The number of cases diagnosed in the second trimester, from weeks 18–22, did not change significantly over the 11-year period (Figure 2).

In 2006 and 2007, 118 cases of T18 or T13 (88 cases of T18 and 30 cases of T13) were diagnosed prenatally or postnatally. Fourteen women had invasive testing performed for other indications and did not undergo first-trimester screening. The remaining 104 women underwent first-trimester screening. In 11 cases no risk assessment was performed, as major fetal malformations were detected when performing the nuchal translucency scan and termination of pregnancy was chosen. Therefore, 93 women had a first-trimester risk assessment performed. Figure 3 gives an overview of the screening results.

The detection rate of T18 and T13 for women participating in first-trimester risk assessment was 78.8% (95% CI, 71.0–86.7%) (82 detected out of 104). The overall detection rate of T18 and T13 in Denmark in 2006 and 2007 was 90.4% (95% CI, 84.7–96.1%) (94 out of 104) when including the fetuses diagnosed in the second trimester. Eight women, who were first-trimester screen negative, did not have a second-trimester scan performed: in seven cases because CVS was performed for other indications despite the negative screening result, and in one case an anomaly scan was not offered (as in 2006 it was not offered by all obstetric departments in Denmark). If these eight cases are excluded, because we do not know if a second-trimester scan would have detected malformations, the overall detection rate when performing first-trimester risk assessment and second-trimester anomaly scans would have been 97.9% (95% CI, 95.1–100%) (94 out of 96).

DISCUSSION

The introduction of a new screening strategy for T21 in Denmark has resulted in a significant increase in the number of T18 and T13 fetuses diagnosed before week 18 and a significant decrease in the number of infants born with T18 or T13. Importantly, the introduction of the new screening strategy has also resulted in a marked decrease in the number of diagnostic invasive tests. From 2000 to 2006, the yearly number of invasive diagnostic procedures in Denmark thus decreased by more than 50%[10].

Several authors have reported on the performance of first-trimester combined screening for T18 and T13. In 2002, Spencer and Nicolaides derived a combined risk algorithm for T18/T13 with a predicted detection rate of 95% using the risk cu t-off of 1 : 150[15]. We found a first-trimester detection rate for T18 and T13 of 78.8% using the same algorithm and cut-off. Overall, it seems that detection rates observed in series prospectively collected are not as high as those expected based on modeled datasets[7,16,17]. In a large, prospectively collected Danish cohort, Kirkegaard *et al.* reported a 73% detection rate of T18 or T13 (cut-off 1 : 150 for T18/T13 and cut-off 1: 300 for T21)[18]. The differences found in detection rates for T18 and T13 in available reports may be explained by the use of different risk algorithms, different cut-off values and different study designs. Overall, however, the available studies show that it is possible to detect the vast majority of fetuses with T18 or T13 when screening for T21 using the first-trimester combined screening strategy.

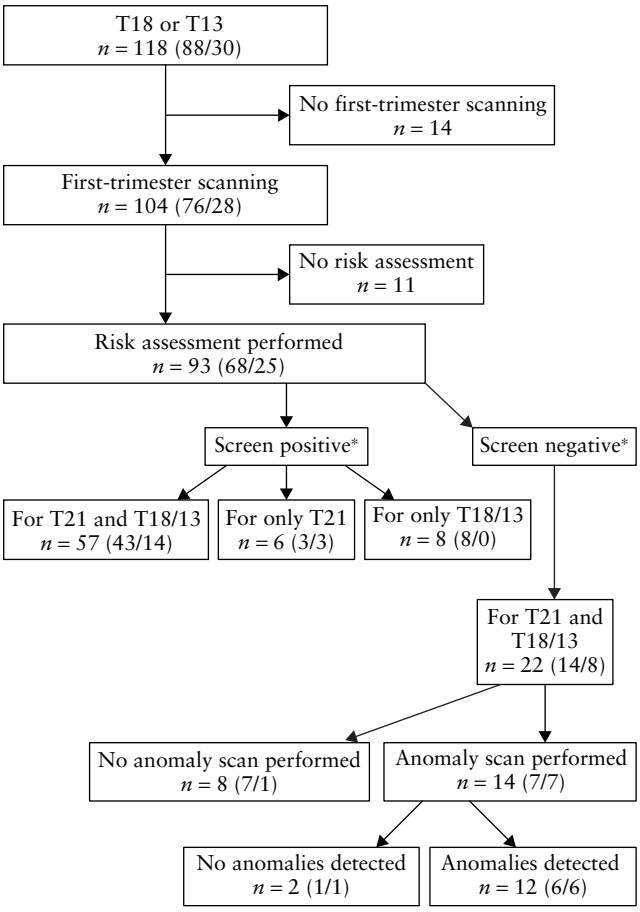

Figure 3 Total number of cases with trisomy 18 (T18) or trisomy 13 (T13) in 2006 and 2007. Values shown in parentheses represent: number of T18 cases/number of T13 cases. *Screen positive defined as T21 risk > 1 : 300 and T18/T13 risk > 1 : 150 at first-trimester screening.

One could argue that screening for T18 and T13 in the first trimester is not necessary because the majority of fetuses will either die *in utero* or be detected if a second-trimester scan is performed. Recently, Morris *et al.* established gestational-age-specific loss rates for T18 and T13[1]. These rates are not as high as previously reported by Snijders *et al.*[14]. Approximately 72% of fetuses with T18 and 49% with T13 will be lost from week 12 to term. Detection rates for T18 and T13 in the second trimester, determined using ultrasound scans, have been reported to be 80–86% for T18 and 90–100% for T13[19]. However, no algorithm for the second-trimester ultrasound screening is available and the screen-positive rate is not known. As the overall screen-positive rate only increases slightly (by 0.1%[11]) when including the screening algorithm for T18 and T13 in the first-trimester screening program for T21, it is considered acceptable to include screening for T18 and T13 in the screening program. Most women prefer screening to be performed early in pregnancy[20] and should the fetus be diagnosed with T18 or T13, the parents can opt for a safer termination of the pregnancy in the first trimester[21].

In the present study, the estimated incidences at delivery of T18 (2.5 per 10 000) and T13 (1.6 per 10 000) in Denmark between 1997 and 2007, taking the fetal loss rate into account, were very similar to those reported by other authors, of 1.25–2.8 per 10 000 for T18 and 0.5–2.0 per 10 000 for T13[22]. The differences in the rates are probably because of the small study size and diversity in rate calculation; some include and others exclude prenatally diagnosed fetuses and pregnancy terminations, and different fetal loss rates are used (if taken into account at all), when calculating the rates.

As expected, we found that the median age of mothers with a T18 or T13 fetus/infant was higher than the median age of mothers who did not carry a fetus with T18 or T13. We found the median survival time to be 5 days and 4.5 days, respectively, for T18 and T13. This confirms results from previous studies reporting median survival times of 3–14.5 days and 2.5–8.5 days, respectively[22].

The main strength of our study was that it comprised a large national dataset collected in a homogeneous low-risk population. Local quality assurance of the ultrasound scans was performed via The Fetal Medicine Foundation (www.fetalmedicine.com), and the biochemistry departments were accredited by EN15189. Our national cytogenetic register compiles results from all chromosome analyses and is considered to be almost 100% complete. There could be potential bias when comparing data collected in one register over a 10-year period; however, no major changes of reporting into the register occurred during this time period. Our prenatal screening information on cases with T18 or T13 in 2006 and 2007 was considered complete because we were able to find detailed screening information on all cases registered in the national cytogenetic register.

A limitation of our study was that although the data were prospectively registered, our main objective was to look retrospectively at the gestational age at which T18 and T13 were diagnosed throughout the 11-year period. Therefore, we did not have information on the overall first-trimester screen-positive rate for T18/T13. However, we do not expect it to vary from the very low false-positive rates (< 0.5%) expected and reported in other study populations[11,15]. In Denmark the screen-positive rate for T21 using the cut-off of 1 : 300 is low (3.5% in 2006)[10]. Therefore, a small increase in the screen-positive rate, when including screening for T18 and T13 in the program, is fully acceptable.

To the best of our knowledge this is the first report, using national data, to describe how first-trimester screening for T21 in routine clinical practice has changed the gestational age at detection of T18 and T13. In Denmark, more than 90% of pregnant women participate in the screening program[13] and, as expected, we found that the number of fetuses diagnosed late in pregnancy or infants born with either T18 or T13 decreased significantly from 1997 to 2007. We consider quality control of a screening program as very important. It is essential in order to update pregnant women, sonographers and doctors on the screening performance and is required to specify where improvement is needed.

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REFERENCES

1. Morris JK, Savva GM. The risk of fetal loss following a prenatal diagnosis of trisomy 13 or trisomy 18. *Am J Med Genet A* 2008; **146**: 827–832.
2. Goldstein H, Nielsen KG. Rates and survival of individuals with trisomy 13 and 18. Data from a 10-year period in Denmark. *Clin Genet* 1988; **34**: 366–372.
3. Parker MJ, Budd JL, Draper ES, Young ID. Trisomy 13 and trisomy 18 in a defined population: epidemiological, genetic and prenatal observations. *Prenat Diagn* 2003; **23**: 856–860.
4. Rasmussen SA, Wong LY, Yang Q, May KM, Friedman JM. Population-based analyses of mortality in trisomy 13 and trisomy 18. *Pediatrics* 2003; **111**: 777–784.
5. Spencer K. Aneuploidy screening in the first trimester. *Am J Med Genet C Semin Med Genet* 2007; **145C**: 18–32.
6. Kagan KO, Etchegaray A, Zhou Y, Wright D, Nicolaides KH. Prospective validation of first-trimester combined screening for trisomy 21. *Ultrasound Obstet Gynecol* 2009; **34**: 14–18.
7. 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; **25**: 221–226.
8. 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-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med* 2005; **353**: 2001–2011.
9. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998; **352**: 343–346.
10. Ekelund CK, Jorgensen FS, Petersen OB, Sundberg K, Tabor A. Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study. *BMJ* 2008; **337**: a2547.
11. 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; **23**: 1968–1975.
12. Kagan KO, Wright D, Maiz N, Pandeva I, Nicolaides KH. Screening for trisomy 18 by maternal age, fetal nuchal translucency, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 2008; **32**: 488–492.
13. Ekelund CK, Andersen HJ, Christensen J, Ersbak V, Farlie R, Henriques C, Holmskov A, Jensen LN, Jorgensen FS, Hessellund A, Larsen T, Olesen AW, Pedersen OB, Poulsen H, Ramb J, Skibsted L, Skovbo P, Sommer S, Sperling L, Sundberg K, Juul SV, Zingenberg H, Tabor A. [Down's syndrome risk assessment in Denmark – secondary publication]. *Ugeskr Laeger* 2010; **172**: 1759–1761.
14. Snijders RJ, Holzgreve W, Cuckle H, Nicolaides KH. Maternal age-specific risks for trisomies at 9–14 weeks' gestation. *Prenat Diagn* 1994; **14**: 543–552.
15. Spencer K, Nicolaides KH. A first trimester trisomy 13/trisomy 18 risk algorithm combining fetal nuchal translucency thickness, maternal serum free beta-hCG and PAPP-A. *Prenat Diagn* 2002; **22**: 877–879.
16. 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 screening: detection of aneuploidies other than Down syndrome. *Obstet Gynecol* 2007; **110**: 651–657.
17. Jaques AM, Halliday JL, Francis I, Bonacquisto L, Forbes R, Cronin A, Sheffield LJ. Follow up and evaluation of the Victorian first-trimester combined screening programme for Down syndrome and trisomy 18. *BJOG* 2007; **114**: 812–818.
18. Kirkegaard I, Petersen OB, Uldbjerg N, Torring N. Performance of first-trimester combined screening for trisomy 13 and 18 with the double test taken at a gestational age of 8 + 0 to 13 + 6. *Prenat Diagn* 2009; **29**: 582–587.
19. Shipp TD, Benacerraf BR. Second trimester ultrasound screening for chromosomal abnormalities. *Prenat Diagn* 2002; **22**: 296–307.
20. de Graaf IM, Tijmstra T, Bleker OP, van Lith JM. Womens' preference in Down syndrome screening. *Prenat Diagn* 2002; **22**: 624–629.
21. Bartlett LA, Berg CJ, Shulman HB, Zane SB, Green CA, Whitehead S, Atrash HK. Risk factors for legal induced abortion-related mortality in the United States. *Obstet Gynecol* 2004; **103**: 729–737.
22. Vendola C, Canfield M, Daiger SP, Gambello M, Hashmi SS, King T, Noblin SJ, Waller DK, Hecht JT. Survival of Texas infants born with trisomies 21, 18, and 13. *Am J Med Genet A* 2010; **152A**: 360–366.

A prospective study evaluating the performance of first trimester combined screening for trisomy 21 using repeated sampling of the maternal serum markers PAPP-A and free β-hCG

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Short title: Screening for trisomy 21 using a double set of maternal serum markers.

Keywords: combined first trimester screening, maternal serum markers, free beta-hCG, PAPP-A, nuchal translucency, screening for trisomy 21

Abstract

Objective: To prospectively evaluate the screening performance of first trimester combined screening for trisomy 21 using a double set of the biochemical markers PAPP-A and free β-hCG

Methods: Three fetal medicine departments in Denmark participated in the study. Screening for trisomy 21 was set up as a two-step approach with blood sampling performed before the nuchal translucency scan (early sample). A second blood sample was collected at the time of the nuchal translucency scan (late sample). PAPP-A and free β-hCG were measured on both the early and the late samples. Age standardised detection and false positive rates for different screening protocols were produced.

Results: We collected two blood samples in 27 pregnancies affected by trisomy 21 and in 3891 control pregnancies. The early samples were taken between gestational week 8+0 up to week 13+6, and the late samples between week 11+3 up to week 14+6. The median interval between the samples was 17 days (range 1-40 days). We found a significantly better ($P<0.05$) estimated screening performance when using early sampling vs. late sampling. With a risk cut-off of 1 in 100, at the time of the risk assessment, the estimated detection and false positive rates were 91% (95% CI: 81-98%) and 1.6 % (95% CI: 1.3-2.0%) respectively. Better estimated performance was achieved with the use of the double set of markers; detection rate 93% (95%CI: 85-99%) and false positive rate 1.7% (95% CI 1.4-2.0%), but this was not significantly different from the early sample protocol ($P>0.5$).

Conclusion: Using early sampling with measurement of PAPP-A and free β-hCG in the combined first trimester screening can optimise screening performance for trisomy 21. Using a double set of the maternal serum markers has the potential to further improve screening performance.

Introduction

Screening for trisomy 21 using the first trimester combined screening strategy is now offered to pregnant women in many countries. The screening test combines the maternal age related risk for trisomy 21 with the nuchal translucency thickness measured by ultrasound in pregnancy week 11 to 14 and analysis of two maternal serum markers, Pregnancy Associated Plasma Protein-A (PAPP-A) and free beta human Chorionic Gonadotrophin (free β-hCG), in week 8 to 14. Prospective studies have demonstrated that the first trimester combined screening test has a detection rate about 90 % for a 5% false positive rate[1-3].

It is well established that there is a temporal variation of the biochemical markers across the first and second trimester in cases with trisomy 21 [4-7]. The mean log multiple of the median (MoM) free β-hCG increases linearly with gestation between 8 and 13 weeks in trisomy 21 pregnancies, and thus the discrimination between affected and unaffected pregnancies is more pronounced late in the first trimester. This is in contrast to PAPP-A where the maximum discrimination between trisomy 21 and unaffected pregnancies occurs at 9-10 weeks as described by Wright et al. [5]. The screening performance of the first trimester combined screening for trisomy 21 is therefore dependent on gestational age at blood sampling. Many centres have established one-stop clinics for assessment of risk (OSCAR) [8], where blood sampling, ultrasound assessment and counselling are done on the same day, usually around pregnancy week 12-13. It has been suggested that because of the greater discrimination power of PAPP-A, blood sampling at 9-11 weeks would provide a better overall screening performance [4-6]. Two studies have prospectively evaluated this concept and have found that screening performance is better when the blood sample is taken early in the first trimester [9,10]. An alternative approach to further improve performance would be to measure PAPP-A early at 9 weeks and free β-hCG late at 12 weeks or to measure both markers at 9 and 12 weeks of gestation.

The objective of this study was to evaluate prospectively, how screening performance is affected by time of sampling within the same pregnancy and to evaluate if a double set of the maternal serum markers PAPP-A and free β-hCG can improve the screening performance of first trimester combined screening for trisomy 21.

Material and methods

The study was performed at three centres of Fetal Medicine in Denmark (Rigshospitalet, Hvidovre and Skejby Hospital) between 01 June 2008 and 31 September 2010. At all three centres the screening was set up as a two-step approach where blood sampling and the nuchal translucency scan were performed on separate occasions.

During the study period the first blood sample (referred to as the *early sample*) was taken at the hospitals or by the general practitioners. When the women came for the nuchal translucency scan, they were asked for permission to provide a second blood sample (referred to as the *late sample*) for measurement of PAPP-A and free β-hCG. We aimed at collecting the samples at the same time as the nuchal scan. In some cases this was not possible due to logistic problems and then the women were asked to return for the additional blood sampling before week 14. The results of the late sample were neither disclosed to the participants, nor to the sonographers.

We included only singleton pregnancies in the study population and excluded pregnancies with chromosomal abnormalities other than trisomy 21 including trisomy 21 mosaicism, twin and higher order pregnancies, and those in which the second blood sample for some reason was taken later than 14+6 weeks.

Ultrasound scanning was performed in the three hospitals by sonographers all certified by the Fetal Medicine Foundation (FMF) in London, UK. The crown-rump length (CRL) was measured to determine gestational age, and nuchal translucency thickness was measured according to the FMF guidelines, from 11+2 to 13+6 weeks of gestation (CRL 45-84 mm). All data were registered in the local Astraia databases (Astraia Gmbh) for each participating centre. All risk assessments in the three centres were based on maternal age, first (early) blood sample values of PAPP-A and free β-hCG and nuchal translucency thickness. No other ultrasound markers were included in the risk assessment. Women were offered an invasive diagnostic test, when the combined risk was 1:300 or higher at the time of testing.

In two centres (Rigshospitalet and Hvidovre Hospital) the second (late) blood samples were immediately analysed together with routine serum samples, using the Brahms Kryptor. At the third centre (Skejby) the samples were collected as part of a pregnancy biobank. Samples were frozen to -80°C within 2 hours of collection, thawed and analysed using the Brahms Kryptor when study inclusion had stopped.

Information about the pregnancy including gestational age determined by CRL at the nuchal translucency scan, maternal weight, ethnicity, smoking, method of conception, concentration of the serum markers and date of sampling for the early blood test was obtained from the local Astraia databases. This information was taken into account when converting exact values of the two set of serum markers into MoM values. Results of prenatal and postnatal karyotypes were obtained from the Departments of Clinical Genetics at Rigshospitalet (covering Rigshospitalet and Hvidovre Hospital) and Skejby Hospital, and cross-checked with the Danish Central Cytogenetic Register (DCCR).

The study was approved by the Ethical Committee of the Capital Region of Denmark (H-A-2008-37) and the Data Protection Agency in Denmark (2008-41-2928).

Statistics

We defined *four* first trimester screening protocols. All protocols used maternal age, nuchal translucency and biochemical markers in different combinations as shown in table 1. Autocorrelation and cross correlation functions based on the current data set for the two markers were used to define the multivariate Gaussian distributions for combinations of log MoM PAPP-A and log MoM free β hCG from the two samples. Each patient specific risk for trisomy 21 was obtained combining the maternal age related risk and the likelihood ratio using Bayes theorem. The likelihood ratios were obtained from the mixture model for NT [5] and fitted Gaussian likelihood ratios for maternal serum free β-hCG and PAPP-A log MoM values from the early and late samples as appropriate[4]. Estimates of screening performance were obtained by first computing likelihood ratios for unaffected and trisomy 21 pregnancies for each pregnancy in the sample. For the unaffected pregnancies, these likelihood ratios were used to obtain a maternal age specific false positive rate for each maternal age from 14 to 50. A weighted average of these maternal age specific false positive rates, with respect to the reference distribution of maternal ages in unaffected pregnancies was then calculated producing an estimate of the false positive rate in the reference population. Similarly, the likelihood ratios for the trisomy 21 pregnancies were used to compute estimates of maternal age specific detection rates. A weighted average of these, with respect to the reference maternal age distribution of trisomy 21 pregnancies in the reference population was then calculated producing an estimate of the detection rate in the reference

population. Confidence intervals for the detection and false positive rates were obtained using bootstrapping. The reported screening performance is based on risk at the time of screening and standardised to maternal age distribution of England and Wales 2002[11].

Results

We collected two blood samples in 4,716 pregnancies. After exclusion of pregnancies with chromosomal abnormalities other than trisomy 21 (including trisomy 21 mosaicism), twin and higher order pregnancies, and those in which the second blood sample was taken later than 14+6 weeks, the study population consisted of 27 pregnancies affected by trisomy 21 and 3891 control pregnancies defined as having either a normal prenatal chromosome analysis or no abnormal karyotype registered postnatally. Details of maternal characteristics in the total study population are shown in Table 2 according to the three centres. The early blood samples were taken between 8 weeks +0 days and 13 weeks + 6 days, 72% were taken before 11 weeks + 2 days. The median interval between the two samples was 17 days (range 1-40 days).

Screening performance:

Table 3 gives an overview of detection rates according to fixed false positive rates for the four different screening protocols. We achieved the best screening performance when using markers from two samples with a detection rate as high as 97% for a fixed false positive rate of 3% (protocol 3 and 4). Using both markers from only the early sample provided a detection rate of 95% (protocol 2), compared to a detection rate of 92% when using only the late sample (protocol 1).

We calculated screening performance according to the four screening protocols for different fixed cut-offs (table 4). We found a significant benefit in terms of reduced FPR for early vs. late sampling (protocol 2 vs. protocol 1) using a risk cut of 1 in 100 at the time of screening. The estimated reduction in FPR was 0.52% (95% CI: 0.23% to 0.80%). There was also a trend towards improvement in detection rate which did not reach significance at the 5% level. The estimated increase in detection rate was 2.9% (95% CI: -1.3% to 7.1%).

The best strategy, in terms of estimated performance was the test with all four markers (protocol 4). Compared to early sampling of both markers (protocol 2), using a risk cut-off of 1 in 100 at the time of screening, detection increased by an estimated 1.6% (95% CI: -1.3% to 4.6%), although the evidence of improvement did not achieve significance at the 5% level.

Discussion:

This is the first large prospective study performed to investigate the potential benefits of having access to a double set of maternal serum markers within the first trimester of a pregnancy.

This study confirms that screening performance is affected by time of blood sampling. We found that screening performance is improved when risk calculation is based on an early first trimester sample compared to the performance found when the sample is taken at the time of the nuchal translucency scan. This finding was expected due to previous published data on the biochemical markers' gestational age dependent discriminatory power and in line with two previous studies, which evaluated early blood sampling in the first trimester. Kirkegaard et al found in a register-based study a significantly higher detection rate when the sample was taken before gestational week 10 compared to at or after gestational week 10 (DR 100% vs. 77%)[10]. Borrell et al. found a remarkably reduced false positive rate in their prospective evaluation of the first trimester combined screening programme where mean gestational age at blood sampling was 9.4 weeks[9]. There is now accumulating evidence that performance of the first trimester combined test is improved when the blood sampling is done prior to the nuchal translucency measurement. Since the publication of these two studies extensive work has been made to improve the statistically complicated algorithms used for patient specific risk calculation [4,5]. Screening performance has been improved by development of optimised algorithms and therefore a substantial improvement will be difficult to obtain in any new screening approaches. Moving towards earlier biochemical sampling is however a relatively simple way to optimise the screening system. In England the national screening committee has set a goal of a 90 % detection rate for a 2 % false positive rate [12]. In our data this is possible to achieve by using a cut off of 1 in 100 with early blood sampling (DR 91 %, FPR 1.6%).

Other countries or centres may prefer to improve the detection rate without changing the false positive rate. By having access to two samples (protocol 3 or 4) we found that it is possible to keep the false positive rate below 4% and increase the detection rate up to 97%. Repeated testing between the first and second trimester has been investigated by several authors [4,13-15]. As screening for trisomy 21 has moved toward first trimester combined screening over the last 5 years, it is relevant to investigate repeated testing within the first trimester. To the best of our knowledge only one paper has previously investigated if repeat maternal serum screening within

the first trimester can improve screening performance. Spencer and Cuckle used a data set with 261 paired samples taken between weeks 10+0 to 13+6 from unaffected pregnancies[16]. They established correlation matrixes, investigated biological variability and subsequently modelled the data to describe detection rates in a protocol which included NT measurement and maternal serum markers measured at 10 weeks and 12 weeks. They found a detection rate of 88.6% for a fixed false positive rate of 5%, which was only a 1.3% increase in detection rate compared to using only one sample. We found when using our large data set including also 27 cases of trisomy 21, screening performance to be very high (detection rates 97% for a false positive rate of 3%) when using two samples (protocol 3 or 4). In contrast to Spencer and Cuckle our calculations are based on the mixture model (wright et al. 2008)[17] and the maternal serum biochemistry is adjusted for maternal characteristics as described by Kagan et al 2008[4] which should provide optimised screening performance in itself.

The strength of our study is that it is a large study with prospectively collected data including trisomy 21 cases, and the presented results are not based on modelled data. The data set was collected at three different centres with slightly different ways of handling the samples. Thus the data do not represent a strict study set up, and therefore we believe that our study results reflect what is probably achievable in routine clinical settings. It is possible that an even better screening performance could be achieved if the set up had been stricter with all early samples taken between gestational week 9 and 10. In the available data set about one third of the samples were taken after week 10. Again the study probably does not overestimate the screening performance and the reported results should be achievable in other centres.

Although the data set does contain cases with trisomy 21, the number is still limited. It is possible that the correlation matrixes and the risk calculations can be improved by having access to more data. Thus before repeated testing is offered to pregnant women outside a study set up we do suggest validation of the results in independent data sets. In addition it has to be considered if repeated testing is cost effective by lowering the number of invasive procedures. Many alternative ways of improving the screening performance have been suggested. Assessment of additional first trimester ultrasound markers like the nasal bone, ductus venosus flow and flow across the tricuspid valves have also been found to benefit screening performance [18-20]. Evaluation of these markers however requires training of sonographers and a substantial number of scans are needed

to properly determine the presence or absence of the markers[21,22]. In addition the examination time required for the nuchal translucency scan is increased. The advantages of repeated blood sampling are that no training of staff is required and that it is built on an already established laboratory analysis. It is important to work on improvement of the screening programme from many directions. One centre may find it logistically simple to implement an extra blood sample in the risk calculation, other centres may not be able to establish the offer of early blood sampling, but prefer to train the sonographers to use assessment of additional ultrasound markers in the risk calculation.

In conclusion we have described how the performance of the first trimester combined screening programme is affected by the time of blood sampling. Having access to an early blood sample is superior to sampling at the same time as the nuchal translucency scan. In addition it seems possible to improve the screening performance even more, if repeated blood sampling within the first trimester is performed. Optimisation of screening performance in a screening programme like first trimester combined screening for trisomy 21 which is used world wide is always relevant as even small improvements may lead to a substantial decrease in number of invasive tests performed and subsequently in number of pregnancies affected by procedure related loss.

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Table 1: Investigated screening protocols

Protocol 1	MA + NT + PAPP-A (late sample) + free β-hCG (late sample)
Protocol 2	MA + NT + PAPP-A (early sample) + free β-hCG (early sample)
Protocol 3	MA + NT + PAPP-A (early sample) + free β-hCG (late sample)
Protocol 4	MA + NT + PAPP-A (early and late sample) + free β-hCG (early and late sample)

MA: Maternal age, NT: Nuchal translucency

Table 2: Background information study population in total and by centre

Parameter	Total (n = 3918)	Rigshospitalet (n = 1752)	Hvidovre (n = 990)	Skejby (n = 1176)
Maternal characteristics				
Maternal age (years) (median, IQR)	31 (28-34)	31 (29-35)	31 (28-33)	30 (27-33)
Maternal weight (kg) (median, IQR)	65 (59-72)	64 (59-70)	65 (59-73)	66 (60-74)
Mode of conception				
Spontaneous	3546 (90.5 %)	1569 (89.6 %)	889 (89.8 %)	1088 (92.5 %)
IVF	120 (3.1 %)	67 (3.8 %)	24 (2.4 %)	29 (2.5 %)
ICSI	69 (1.8 %)	34 (1.9 %)	18 (1.8 %)	17 (1.7 %)
Ovulation-induction drugs	168 (4.3%)	76 (4.3 %)	50 (5.1 %)	42 (3.6 %)
Not reported	15 (0.4 %)	6 (0.3 %)	9 (0.9 %)	0
Smoking status				
Non-smokers	3676 (93.8 %)	1658 (94.6 %)	882 (89.1 %)	1136 (96.6 %)
Smokers	147 (3.8 %)	60 (3.4 %)	52 (5.3 %)	35 (3.0 %)
Stopped smoking	82 (2.1 %)	32 (1.8 %)	46 (4.6 %)	4 (0.3 %)
Not reported	13 (0.3 %)	2 (0.1 %)	10 (1.0 %)	1 (0.1 %)
Ethnicity				
Caucasian	3803 (97.1 %)	1714 (97.8 %)	948 (95.8 %)	1141 (97.0 %)
Afro-caribbean	9 (0.2 %)	4 (0.2 %)	2 (0.2 %)	3 (0.3 %)
Asian	34 (0.9 %)	10 (0.6 %)	8 (0.8 %)	16 (1.4 %)
Oriental	37 (0.9 %)	12 (0.7 %)	15 (1.5 %)	10 (0.9 %)
Mixed/other	18 (0.5 %)	7 (0.4 %)	5 (0.5 %)	6 (0.5 %)
Not reported	17 (0.4 %)	5 (0.3 %)	12 (1.2 %)	0
Gestational age at first sample (weeks)				
8+0 - 8+6	500 (12.8 %)	121 (6.9 %)	16 (1.6 %)	363 (3.9 %)
9+0 - 9+6	1027 (26.2 %)	403 (23.0 %)	108 (10.9 %)	516 (43.9 %)
10+0 - 10+6	1143 (29.2 %)	533 (30.4 %)	375 (37.9 %)	235 (20.0 %)
11+0 - 11+6	927 (23.7 %)	486 (27.7 %)	384 (38.8 %)	57 (4.8 %)
12+0 - 12+6	296 (7.6 %)	190 (10.8 %)	105 (10.6 %)	4 (0.3 %)
13+0 - 13+6	22 (0.6 %)	19 (1.1 %)	2 (0.2 %)	1 (0.1 %)
14+0 - 14+6	0	0	0	0
Not reported	0	0	0	0
Gestational age at second sample (weeks)				
8+0 - 8+6	0	0	0	0
9+0 - 9+6	0	0	0	0
10+0 - 10+6	4 (0.1 %)	4 (0.2 %)	0	0
11+0 - 11+6	307 (7.8 %)	148 (8.4 %)	80 (8.1 %)	79 (6.7 %)
12+0 - 12+6	1728 (44.1 %)	749 (42.8 %)	399 (40.3 %)	580 (49.3 %)
13+0 - 13+6	1760 (44.9 %)	767 (43.8 %)	480 (48.5 %)	514 (43.7 %)
14+0 - 14+6	118 (3.0 %)	84 (4.8 %)	31 (3.1 %)	3 (0.3 %)
Number of days between samples				
Median and IQR	17 (12-23)	15 (10-21)	14 (10-17)	24 (21-28)

Table 3: Detection rate (95 % CI) according to screening protocol at different fixed false positive rates

	False positive rate			
	0.5%	**1%**	**2%**	**3%**
Protocol 1 PAPP-A (late sample) + free β-hCG (late sample)	**76%** (61% - 88%)	**83%** (68% - 93%)	**88%** (76% - 96%)	**92%** (82% - 98%)
Protocol 2 PAPP-A (early sample) + free β-hCG (early sample)	**82%** (67% - 92%)	**88%** (75% - 96%)	**92%** (83% - 98%)	**95%** (87% -99%)
Protocol 3 PAPP-A (early sample) + free β-hCG (late sample)	**85%** (71% - 94%)	**90%** (78% -97%)	**94%** (84% - 99%)	**97%** (90% - 99%)
Protocol 4 PAPP-A (early and late sample) + free β-hCG (early and late sample)	**88%** (74% - 96%)	**91%** (79% - 98%)	**94%** (85% - 99%)	**97%** (90% - 99%)

Table 4: Screening performance (detection rates (DR) and false positive rates (FPR)) for different fixed cut offs

	1 in 50		1 in 100		1 in 200		1 in 300	
	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)	DR (%)	FPR (%)
Protocol 1 PAPP-A (late sample) + free β-hCG (late sample)	**83** (70 - 93)	**1.2** (0.9 - 1.4)	**88** (77 - 96)	**2.1** (1.8 - 2.5)	**93** (83 - 99)	**3.9** (3.5 - 4.4)	**94** (86 - 99)	**5.4** (4.9 - 6.0)
Protocol 2 PAPP-A (early sample) + free β-hCG (early sample)	**87** (75 - 96)	**0.9** (0.7 - 1.2)	**91** (81 - 98)	**1.6** (1.3 - 2.0)	**94** (86 - 99)	**2.8** (2.4 - 3.2)	**96** (89 - 99)	**4.0** (3.5 - 4.4)
Protocol 3 PAPP-A (early sample) + free β-hCG (late sample)	**90** (79 - 97)	**1.1** (0.8 - 1.3)	**93** (84 - 99)	**1.9** (1.5 - 2.1)	**97** (90 - 99)	**3.1** (2.7 - 3.5)	**97** (91 - 99)	**4.2** (3.7 - 4.7)
Protocol 4 PAPP-A (early and late sample) + free β-hCG (early and late sample)	**91** (80 - 98)	**1.0** (0.8 - 1.2)	**93** (85 - 99)	**1.7** (1.4 - 2.0)	**96** (89 - 99)	**2.9** (2.5 - 3.3)	**97** (91 - 99)	**4.0** (3.5 - 4.5)

Reference List

(1) Ekelund CK, Jorgensen FS, Petersen OB, Sundberg K, Tabor A. Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study. *BMJ.* 2008;337:a2547.

(2) Kagan KO, Etchegaray A, Zhou Y, Wright D, Nicolaides KH. Prospective validation of first-trimester combined screening for trisomy 21. *Ultrasound Obstet Gynecol.* 2009;34:14-18.

(3) 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;25:221-226.

(4) Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound Obstet Gynecol.* 2008;31:493-502.

(5) Wright D, Spencer K, Kagan KK et al. First-trimester combined screening for trisomy 21 at 7-14 weeks' gestation. *Ultrasound Obstet Gynecol.* 2010;36:404-411.

(6) Cuckle HS, van Lith JM. Appropriate biochemical parameters in first-trimester screening for Down syndrome. *Prenat Diagn.* 1999;19:505-512.

(7) Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. Temporal changes in maternal serum biochemical markers of trisomy 21 across the first and second trimester of pregnancy. *Ann Clin Biochem.* 2002;39:567-576.

(8) 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;20:219-225.

(9) Borrell A, Casals E, Fortuny A et al. First-trimester screening for trisomy 21 combining biochemistry and ultrasound at individually optimal gestational ages. An interventional study. *Prenat Diagn.* 2004;24:541-545.

(10) Kirkegaard I, Petersen OB, Uldbjerg N, Torring N. Improved performance of first-trimester combined screening for trisomy 21 with the double test taken before a gestational age of 10 weeks. *Prenat Diagn.* 2008;28:839-844.

(11) Office for National Statistics. *Birth Statistics. Review of the Registrar General on births and patterns of family building in England and Wales*. *Series FM1*, No. 29–31. Stationery Office: London, 2002.

(12) Screening for Down's syndrome: UK NSC Policy recommendations 2011–2014 Model of Best Practice (/http://fetalanomaly.screening.nhs.uk/standardsandpolicies). 2011.

(13) Palomaki GE, Steinort K, Knight GJ, Haddow JE. Comparing three screening strategies for combining first- and second-trimester Down syndrome markers. *Obstet Gynecol.* 2006;107:367-375.

(14) Wald NJ, Bestwick JP, Morris JK. Cross-trimester marker ratios in prenatal screening for Down syndrome. *Prenat Diagn.* 2006;26:514-523.

(15) Wright DE, Bradbury I. Repeated measures screening for Down's Syndrome. *BJOG.* 2005;112:80-83.

(16) Spencer K, Cuckle HS. Screening for chromosomal anomalies in the first trimester: does repeat maternal serum screening improve detection rates? *Prenat Diagn.* 2002;22:903-906.

(17) Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound Obstet Gynecol.* 2008;31:376-383.

(18) Cicero S, Avgidou K, Rembouskos G, Kagan KO, Nicolaides KH. Nasal bone in first-trimester screening for trisomy 21. *Am J Obstet Gynecol.* 2006;195:109-114.

(19) Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0 to 13+6 weeks of gestation. *Ultrasound Obstet Gynecol.* 2009;33:18-22.

(20) Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. *Ultrasound Obstet Gynecol.* 2009;33:512-517.

(21) Cicero S, Dezerega V, Andrade E, Scheier M, Nicolaides KH. Learning curve for sonographic examination of the fetal nasal bone at 11-14 weeks. *Ultrasound Obstet Gynecol.* 2003;22:135-137.

(22) Maiz N, Kagan KO, Milovanovic Z, Celik E, Nicolaides KH. Learning curve for Doppler assessment of ductus venosus flow at 11 + 0 to 13 + 6 weeks' gestation. *Ultrasound Obstet Gynecol.* 2008;31:503-506.

Title: Screening performance for trisomy 21 comparing first trimester combined screening and a first trimester contingent screening protocol including ductus venosus and tricuspid flow.

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What is already known about this topic: Ductus venosus flow and tricuspid flow may be used in contingent screening protocols to increase screening performance for trisomy 21. There are few prospective studies evaluating the potential in clinical practice.

What does this study add: This is a two centre prospective study which confirms previous results on how first trimester screening performance is inproved when using additional ultrasound markers. The study was partly done as part of a routine clinical set up.

Key words: screening, trisomy 21, risk assessment, ductus venosus flow, tricuspid flow, high or intermediate risk

Abstract:

Aim: To compare the standard first trimester combined risk assessment for trisomy 21 with a contingent screening protocol including tricuspid flow and ductus venosus flow.

Material and method: Women with singleton pregnancies and a first trimester combined risk assessment > 1:1000 were included. They all had additional assessment of the ductus venosus and the tricuspid flow. We compared screening performance in two screening strategies; a) *First trimester combined screening strategy* based on the individual risk results from the routine screening test. b) *Contingent screening strategy* based on a combination of the routine test results and additional ultrasound markers.

Results: We included 917 women in the study, 894 in the euploid group and 23 in the trisomy 21 group. Using a contingent screening strategy resulted in a significant decrease in screen positive rate from 48.3% to 17.7% ($p < 0.001$) in the studied population. There was no statistical difference in detection rate between the two screening strategies..

Conclusion: There is increasing evidence in favour of using additional ultrasound markers as part of contingent screening protocols in the first trimester. We do suggest performing further studies in routine clinical settings to provide validation of the available risk algorithms.

Introduction:

Screening for trisomy 21 in the first trimester by maternal age, nuchal translucency thickness and the biochemical markers PAPP-A and free β-hCG is now a well established offer to all pregnant women in Denmark. This first trimester combined screening strategy is associated with a detection rate about 90% for a false positive rate of 5%[1,2]. Several authors have studied ductus venosus flow and tricuspid flow as additional ultrasound markers for trisomy 21[3-15]. Fetuses with trisomy 21 more often have abnormal ductus venosus blood flow and/or abnormal tricuspid flow (tricuspid regurgitation) compared to euploid fetuses. The performance of the screening programme can be increased by including these markers in the risk assessment[8,9]. As assessment of the ductus venosus blood flow and tricuspid flow is time consuming and requires appropriately trained sonographers it has been suggested to reserve these examinations for a sub-group of pregnancies with an intermediate risk (between 1:50-1:1000) after combined fetal nuchal translucency measurement and biochemical screening. If this contingent protocol is used, a detection rate > 95% for a false positive rate of less than 3% is achievable according to previously published reports[2,8,9].

The aim of our study was to assess the ductus venosus blood flow and the tricuspid flow in a high and intermediate risk population (risk > 1:1000) and evaluate the markers' possible impact on screenng performance for trisomy 21, by comparing detection and screen positive rates in our study population achieved by either the routine first trimester combined screening strategy (not including the tricuspid and ductus venosus flows) or by a contingent screening strategy, where information on tricuspid and ductus venosus flow was included in the assessment.

Material and method:

This was a prospective study performed in two large Fetal Medicine Centres in Denmark; Copenhagen University Hospital, Rigshospitalet and Aarhus University Hospital, Skejby. Both centres serve a large low risk pregnant population and perform around 5-6000 nuchal scans per year. The pregnant women are offered the first trimester combined screening test based on maternal age, nuchal translucency measurement and maternal biochemistry (PAPP-A and free β-hCG), and both centres use the software system Astraia, version 1.21 (www.astraia.com) to calculate individual risks. The cut off used for referral to invasive testing is 1:300 at time of screening.

At Copenhagen University Hospital, Rigshospitalet the study was conducted between 1 September 2009 and 30 September 2010. A first trimester combined risk assessment is routinely offered. The blood test is usually taken between gestational week 9 and 11 and the nuchal scan is accordingly performed between week 11+2 and 14+0 by FMF certified sonographers. Women given a combined risk assessment above 1:1000 were informed by the sonographers about the study and if informed consent was achieved they were then rescanned immediately or an appointment was made for an extra scan before gestational week 14 depending on the woman's wish. The examination of the Doppler flows was performed by one investigator (CKE) certified by FMF to perform the ductus venosus and tricuspid flows. No recalculation of the risk for trisomy 21 was performed following assessment of the Doppler flows, but the women were informed that in case the fetus had either abnormal ductus venosus or tricuspid flow they would be offered an invasive diagnostic test (chorionic villus sample or amniocentesis) for reassurance and an additional assessment of the fetal heart at 15 and/or 21 weeks of gestation.

At Skejby University Hospital assessment of the Doppler flows are performed as part of the routine first trimester screening service in special cases and women are given written information about this before the ultrasound scan. In this centre the blood test is also performed before the nuchal scan, and thus the combined risk assessment result is available immediately after the nuchal assessment has been performed. The sonographers, who are all certified by the Fetal Medicine Foundation, aimed to assess and record the ductus and tricuspid flows on women with a combined risk assessment above 1:1000. Assessment of the ductus and tricuspid flows was performed immediately after the nuchal assessment as the calculation of the routine combined risk was done by the sonographers with the woman in the ultrasound scan room. During the study period the first trimester combined risk assessment based on the blood test and the nuchal translucency measurement was not recalculated using the two extra ultrasound markers. Data for this project was retrieved from the local Astraia database between 1st January 2009 and 31 December 2010. Follow up after flow assessment was the same as performed at Rigshospitalet.

In both centres the FMF protocol for assessment of ductus venosus flow and tricuspid flow was used. This method has previously been described by Maiz et al. and Kagan et al[8,9]. We thus recorded an abnormal ducus venosus flow when finding a persistent negative a-wave (reversed flow during atrial contraction), and an abnormal tricuspid flow when regurgitation in the systole was > 60 cm/sec. Women with singleton and multiple pregnancies were initially asked to participate in the study, but it was decided to exclude multiples in the current study population. We also excluded women for whom the ultrasound markers were measured after week 14+5.

The local Departments of Clinical Genetics provided data on all prenatally and postnatally performed cytogenetic analyses in the study population. To verify that no deliveries of infants with trisomy 21 had taken place at other hospitals in Denmark a cross check was made with the Danish Central Cytogenetic Register.

We compared screening performance in the study population for two screening strategies:

1) *The first trimester combined screening strategy* based on the individual risk results provided to each participant in the study as part of their routine screening for trisomy 21 (maternal age, nuchal translucency thickness and maternal biochemical markers). Women with a risk $\geq$ 1: 300 were considered screen positive.

2) *A contingent screening strategy* as outlined in figure 1 based on a combination of the first trimester combined screening results and the additional ultrasound markers. Screen positive in this contingent screening strategy was defined as women with a risk $\geq$ 1:50 based on the combined screening and in addition women with a risk between 1:50-1:1000 and either an abnormal tricuspid flow, an abnormal ductus venosus flow or both abnormal.

Comparison of the two screening strategies was done using McNemars test. Statistical evaluation and analyses were performed using the statistical software programme SPSS18. A p-value < 0.05 was considered significant.

The study was approved by the Ethic Committee System in Denmark (H-C-2009-016) and the Danish Data Protection Agency (2009-41-3430).

Results:

Study population

In the study periods a total of 15 321 first trimester risk assessments were performed in the two centres. 2330 fetuses had a risk assessment > 1:1000 (15.2%). In total 1085 women eligible for inclusion in the study had at least one of the two ultrasound markers recorded (46.6% (61% at Rigshospitalet and 38% at Skejby)). After exclusion of multiples and the 22 cases with chromosomal abnormalities other than trisomy 21 our study population consisted of 917 women/fetuses. The flow charts for inclusion for each centre are shown in figure 2a and 2b. A total of 23 cases with trisomy 21 were observed in the study population (trisomy 21 group) and 894 pregnancies with a known normal prenatal karyotype or with no registered abnormal karyotype postnatally (euploid group). The characteristics of the study population according to centre are shown in table 1.

First trimester markers

Table 2 displays the distribution of first trimester fetal nuchal translucency thickness, maternal serum free beta hCG and PAPP-A values, risk assessments and results of the tricuspid flow and ductus venosus flow in the group with trisomy 21 and in the group with normal karyotype.

Table 3 shows results of the tricuspid flow and ductus venosus flow assessments according to the combined risk assessment in the euploid group and in the trisomy 21 group, respectively.

Evaluation of two screening strategies

In our study population a total of 48.3% (443/917) had a risk at or above 1:300 based on the combined screening test where tricuspid flow and ductus venosus flow were not taken into account in the risk assessment. All 23 cases with trisomy 21 had a first trimester combined risk > 1:300, and would thus have been detected by the routine *first trimester combined screening strategy* (table 2).

Using a contingent *screening strategy* (figure 1), where screen positives are defined as having either a risk assessment $\geq$ 1: 50 (134 in our population) or a risk assessment between 1:51 and 1:1000 and either abnormal tricuspid or abnormal ductus flow (28 in our population) revealed a screen positive rate of 17.7% (162/917). The difference in screen positive rate between the two screening strategies (48.3% vs. 17.7%) was statistically significant ($p< 0.001$).

Twenty of the 23 women in the trisomy 21 group had a risk $\geq$ 1:50. Of the 3 women with a risk below 1:50, one had an abnormal ductus venosus flow, and would thus in a contingent screening strategy be considered screen positive, whereas the last two had normal tricuspid and normal ductus venosus flows, and would therefore in a contingent screening situation be reassured and classified as screen negative (table 3). Thus using a contingent screening strategy including tricuspid and ductus venosus flow would in our studied population lead to a decrease in detection from 100% (23/23) to 91.3% (21/23), although not statistically significant ($p=0.48$).

Discussion:

We found that inclusion of the markers in a contingent screening set up can reduce the number of women who are screened positive by the combined screening strategy. This is in accordance with a number of reports from similar studies[8,9,11,16-18]. In the majority of the published work the study populations are small sub populations often with high risk for trisomy 21. The two major studies from the Fetal Medicine Foundation however provide good evidence for the rationale of assessing additional markers in a contingent screening set up, with only about 15% of the total population needing assessment of these additional markers[8,9]. Kagan et al and Maiz et al. found that inclusion of the additional markers would, in a low risk population result in a detection rate of 96% for a screen positive rate of 2.6%. If our population based screen positive rate was fixed at 5%, the total size of our population would have been 8860 women (443/8860= 5%). Based on our results and using a contingent strategy, we would expect a decrease in screen positive rate to 1.8% (162/8860). The detection rate in a general population cannot be exactly derived from our study, as it only included women with high and intermediate risk. A significant difference in detection rate between the two screening strategies is however not to be expected, since the number of screen negative cases in a low risk population (risk < 1:1000) is limited. Further in our study population with risks > 1:1000 where the majority of trisomy 21 feuses are found there was no significant difference in detection rate between the two screening strategies.

There remains a need for studies on data collected as part of a routine clinical setting using the available risk algorithms. Munez Cortez et al. have recently published a prospective study evaluating a contingent screening strategy similar to our study[18]. They found a possible reduction in screen positive rate from 3.0 % to 1.3-1.8% without change

in the detection rate, but also concluded that in their population the contingent screening strategy was not practical, as they only managed to perform assessment of the ultrasound markers in less than half of the women with an intermediate risk mainly due to logistic problems.

It is a challenge to introduce additional ultrasound markers in a screening set up. A contingent screening strategy can restrict the examinations to be performed either in specialised centres or by few specially trained sonographers in each centre. This seems appropriate as there have been concerns about the markers' implementation in clinical practice as a certain level of expertise is required for a proper assessment[19]. It has been estimated that a sonographer needs to do 80-120 examinations to be able to perform the evaluations correctly on a persistent basis[20].

Our study has some limitations in the design. The study population is of limited size, and restricted to those with high and intermediate risk by the combined risk assessment. Therefore we cannot provide population based prevalence of abnormal flows. The study was performed with a different set up in the two participating centres. As one centre was strictly investigator driven, this centre provided a dataset where almost all participants had assessments of tricuspid and ductus venosus flows. The other centre represents data from a set up where assessment of the flows was performed as part of routine practice although results of the additional markers were not included in the risk assessment. In the routine screening set-up, where 30 minutes are provided to perform scanning and counselling, often only one of the flows was recorded or neither of them were assessed in the women eligible for inclusion. This could potentially have affected the results. But since our results are in agreement with results published by other groups, a possible effect does not seem

to be of any major importance. We found a low uptake rate (38%) at Skejby Hospital as we chose to include all possible participants in the study, knowing that assessment of the flows at the beginning was done less frequently than later in the study period where a much larger proportion of eligible women had assessment of the flows performed. Again our results reflect daily practice where new initiatives are impossible to implement immediately.

We chose to use the FMF criteria in the assessment of the ductus venosus flows categorising them as either normal (positive or absent a-wave) or abnormal (reversed a-wave). Other authors have suggested assessing the ductus venosus flow as a continuous variable using the pulsatility index measurement[21-23]. Borell and Timmerman both found better detection rates when including the ductus venosus pulsatility index in the risk assessment compared to using the a-wave assessment. Further studies are needed to follow up on performance using this approach for assessment of the ductus venosus.
We did not in this study use the available algorithm included as part of the risk programme in the Astraia software. As stressed by Kagan et al, there is still a need for further independent validation of this algorithm[8] . However as we gain experience with additional large datasets it is advisable to use the developed risk calculation programmes as tricuspid flow and ductus venosus flow are dependent on factors like fetal NT, maternal weight and smoking[20]. Only by using these programmes, where complicated statistical adjustment can be performed, is it possible to give the pregnant women optimised individual risk assessments.

In conclusion there is increasing evidence in favour of using tricuspid flow and ductus venosus flow assessments in the screening programme for trisomy 21, and using the markers in a contingent strategy seems appropriate. As the majority of published studies have been performed in highly specialised fetal medicine centres, it is however still important to be cautious when introducing a new concept for routine use. Further studies performed in routine clinical settings which provide validation of the available algorithms are needed before we would consider using the markers routinely in our national screening programme in Denmark.

Acknowledgement

We sincerely thank all the women and their fetuses participating in the study and all sonographers performing the ultrasound scans for the project. We especially acknowledge Benedicte Stavnstrup and Mette Qvortrup Holsting for their particular help in preparing the study set up at the two centres, and the sonographers and midwife Anette Jaeger at Rigshospitalet for their help with recruitment of the participants. We would also like to thank Dr. Susanne Kjaergaard, Department of Clinical Genetics, Rigshospitalet for providing cytogenetic follow up on all participants from Rigshospitalet. In addition we thank Jan Hansen, Danish Central Cytogenetic Register for cross checking the data with national cytogenetic information.

Table 1: Characteristics of the study population

Charateristics	Rigshospitalet n=483	Skejby n=434	Both centres n=917
Maternal age (years)*[median, range]*	33 (18-46)	34 (20-46)	34 (18-46)
Maternal weight (kg) *[median, range]*	64 (44-120)	66 (40-122)	65 (40-122)
Spontaneous conception (%)	407 (84.4)*	364 (84.0)*	771 (84.3)
Non smokers (%)	456 (94.6)*	409 (94.2)	865 (94.4)
Caucasians (%)	467 (96.9)*	407 (93.8)	874 (95.4)
GA at nuchal translucency scan (days) *[median, range]*	90 (78-99)	91 (79-99)	90 (78-99)
GA at measurement of flow (days) *[median, range]*	92 (78-103)	91 (79-99)	91 (78-103)

* one woman with missing information

Table 2: First trimester screening markers and trisomy 21 risk in the high and intermediate risk population according to karyotype

First trimester markers	**euploid group n=894**	**trisomy 21 group n=23**
Nuchal translucency measurement (mm) (mean)	2.1	3.9
PAPP-A MoM (mean)	0.65	0.43
Free beta hCG MoM (mean)	1.42	1.83
Risk assessments		
≥ 1:50 (n, %)	114 (12.8)	20 (87.0)
1:51-1:100 (n, %)	78 (8.7)	1 (4.3)
1:101-1:300 (n, %)	228 (25.5)	2 (8.7)
1:301-1:1000 (n, %)	474 (53.0)	0 (0)
Additional markers		
Tricuspid flow measured	**n=818**	**n=21**
abnormal (n, %)	26 (3.2)	9 (42.9)
normal (n, %)	792 (96.8)	12 (57.1)
Tricuspid flow not measured or inconclusive	**n=76**	**n=2**
Ductus venosus flow measured	**n=818**	**n=20**
abnormal (n, %)	14 (1.7)	5 (25.0)
normal (n, %)	804 (98.3)	15 (75.0)
Ductus venosus flow not measured or inconclusive	**n=76**	**n=3**

Table 3: Tricuspid flow and ductus venosus flow assessment according to risk groups in the euploid group and trisomy 21 group respectively

		TF/DV	TF/DV	TF/DV	TF/DV	TF/DV
	Risk groups	abn/abn	abn/norm **or** abn/incon	norm/abn **or** incon/abn	norm/norm	norm/incon **or** incon/norm
euploid group	≥ 1:50	0	6	7	77	24
	1:51-1:100	0	3	1	55	19
	1:101-1:300	0	9	4	182	33
	1:301-1:1000	0	8	2	392	72
trisomy 21 group	≥ 1:50	3	6	1	5	5
	1:51-1:100	0	0	0	1	0
	1:101-1:300	0	0	1	1	0
	1:301-1:1000	NA	NA	NA	NA	NA

TF: Tricuspid flow

DV: Ductus venosus flow

abn: abnormal

norm: normal

incon: Inconclusive or not assessed

NA: not applicable

Reference List

(1) Ekelund CK, Jorgensen FS, Petersen OB et al. Impact of a new national screening policy for Down's syndrome in Denmark: population based cohort study. *BMJ.* 2008;337:a2547.

(2) Nicolaides KH, Spencer K, Avgidou K et al. 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;25:221-226.

(3) Antolin E, Comas C, Torrents M et al. The role of ductus venosus blood flow assessment in screening for chromosomal abnormalities at 10-16 weeks of gestation. *Ultrasound Obstet Gynecol.* 2001;17:295-300.

(4) Borrell A, Martinez JM, Seres A et al. Ductus venosus assessment at the time of nuchal translucency measurement in the detection of fetal aneuploidy. *Prenat Diagn.* 2003;23:921-926.

(5) Faiola S, Tsoi E, Huggon IC et al. Likelihood ratio for trisomy 21 in fetuses with tricuspid regurgitation at the 11 to 13 + 6-week scan. *Ultrasound Obstet Gynecol.* 2005;26:22-27.

(6) Falcon O, Auer M, Gerovassili A et al. Screening for trisomy 21 by fetal tricuspid regurgitation, nuchal translucency and maternal serum free beta-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks. *Ultrasound Obstet Gynecol.* 2006;27:151-155.

(7) Huggon IC, DeFigueiredo DB, Allan LD. Tricuspid regurgitation in the diagnosis of chromosomal anomalies in the fetus at 11-14 weeks of gestation. *Heart.* 2003;89:1071-1073.

(8) Kagan KO, Valencia C, Livanos P et al. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0 to 13+6 weeks of gestation. *Ultrasound Obstet Gynecol.* 2009;33:18-22.

(9) Maiz N, Valencia C, Kagan KO et al. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. *Ultrasound Obstet Gynecol.* 2009;33:512-517.

(10) Matias A, Gomes C, Flack N et al. Screening for chromosomal abnormalities at 10-14 weeks: the role of ductus venosus blood flow. *Ultrasound Obstet Gynecol.* 1998;12:380-384.

(11) Molina Garcia FS, Carrillo Badillo MP, Zaragoza Garcia EA et al. Analysis of secondary ultrasound markers in the first trimester before chorionic villus sampling. *Prenat Diagn.* 2010;30:1117-1120.

(12) Murta CG, Moron AF, Avila MA et al. Application of ductus venosus Doppler velocimetry for the detection of fetal aneuploidy in the first trimester of pregnancy. *Fetal Diagn Ther.* 2002;17:308-314.

(13) Prefumo F, Sethna F, Sairam S et al. First-trimester ductus venosus, nasal bones, and Down syndrome in a high-risk population. *Obstet Gynecol.* 2005;105:1348-1354.

(14) Toyama JM, Brizot ML, Liao AW et al. Ductus venosus blood flow assessment at 11 to 14 weeks of gestation and fetal outcome. *Ultrasound Obstet Gynecol.* 2004;23:341-345.

(15) Zoppi MA, Putzolu M, Ibba RM et al. First-trimester ductus venosus velocimetry in relation to nuchal translucency thickness and fetal karyotype. *Fetal Diagn Ther.* 2002;17:52-57.

(16) Borrell A, Gonce A, Martinez JM et al. First-trimester screening for Down syndrome with ductus venosus Doppler studies in addition to nuchal translucency and serum markers. *Prenat Diagn.* 2005;25:901-905.

(17) Ghaffari SR, Tahmasebpour AR, Jamal A et al. First Trimester Screening for Chromosomal Abnormalities by Integrated Application of Nuchal Translucency, Nasal Bone, Tricuspid Regurgitation, Ductus Venosus Flow Combined with Maternal Serum Free beta-HCG and PAPP-A: A 5-Year Prospective Study. *Ultrasound Obstet Gynecol.* 2011;132.

(18) Munoz-Cortes M, Arigita M, Falguera G et al. Contingent screening for down syndrome completed in the first trimester: a multicenter study. *Ultrasound Obstet Gynecol.* 2011.

(19) Borrell A. Promises and pitfalls of first trimester sonographic markers in the detection of fetal aneuploidy. *Prenat Diagn.* 2009;29:62-68.

(20) Sonek J, Nicolaides K. Additional first-trimester ultrasound markers. *Clin Lab Med.* 2010;30:573-592.

(21) Bilardo CM, Muller MA, Zikulnig L et al. Ductus venosus studies in fetuses at high risk for chromosomal or heart abnormalities: relationship with nuchal translucency measurement and fetal outcome. *Ultrasound Obstet Gynecol.* 2001;17:288-294.

(22) Borrell A, Borobio V, Bestwick JP, Wald NJ. Ductus venosus pulsatility index as an antenatal screening marker for Down's syndrome: use with the Combined and Integrated tests. *J Med Screen.* 2009;16:112-118.

(23) Timmerman E, Oude RK, Pajkrt E et al. Ductus venosus pulsatility index measurement reduces the false-positive rate in first-trimester screening. *Ultrasound Obstet Gynecol.* 2010;36:661-667.

Figure 1: Definition of "contingent screening strategy"

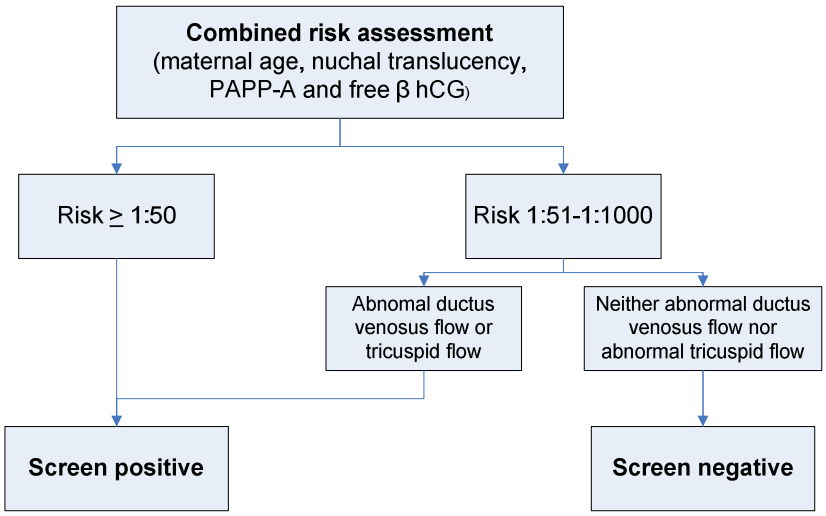

Figure 2a:

Inclusion flow chart Copenhagen University Hospital, Rigshospitalet

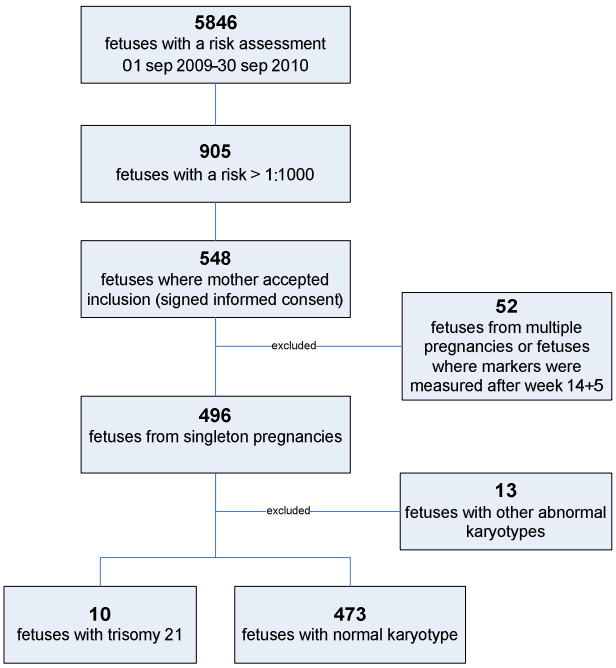

Figure 2b:

Inclusion flow chart Aarhus University Hospital, Skejby