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In the Netherlands prenatal diagnostic testing (PND) for the detection of chromosomal abnormalities has been offered to women of advanced maternal age (AMA or ≥ 36 years) for about four decades. Prenatal screening (PNS) for Down syndrome (DS) was introduced in 1988 with the second-trimester serum screening, combining maternal age, unconjugated oestriol, free β-human chorionic gonadotrophin (fβ-hCG) and alpha-1-fetoprotein. In the last decade the first-trimester combined test (FCT) became available, estimating the individual risk of a woman for DS based on maternal age, fetal nuchal translucency (NT) thickness and concentrations of maternal serum fβ-hCG and pregnancy-associated plasma protein-A (PAPP-A). Up until 2004 pregnant women were screened only on request. Since then, all AMA women were informed of the possibility of PNS, but women aged < 36 years only received information on their explicit request. Since January 2007 the FCT was introduced as population screening for DS in the Netherlands and since May 2010 also screening for Patau syndrome (trisomy 13) and Edwards syndrome (trisomy 18) was allowed using a specific algorithm. Despite the introduction of the FCT the indication AMA for PND is still warranted in the Netherlands and the cost for FCT are only reimbursed for AMA women.

The FCT, a risk assessment test, aims at the highest detection rate (DR), the lowest false-positive rate (FPR) and the lowest odds of being affected given a positive result (OAPR). It is to be expected that the implementation of PNS will reduce the number of invasive diagnostic tests, because women identified with a low risk of DS could avoid invasive testing and the possible procedure-related miscarriage.

The screening performance of the FCT has been reported up to 90% detection of the DS cases at a FPR of 5%. Several factors influence the risk assessment, i.e. a prior pregnancy with trisomy 21, 18 or 13, gestational age, maternal weight, smoking behaviour, in vitro fertilisation (IVF) or intracytoplasmaticsperma injection (ICSI), diabetes, ethnicity, blood loss, the number of fetuses and chorionicity.

In this thesis the effect of implementation of first-trimester screening on the uptake of the FCT and invasive diagnostic tests is described as well as the screening performance of the FCT in its current form and with several adjustments.

Chapter 1 contains a brief general introduction on the organisation and the introduction of prenatal screening in the Netherlands. Further it describes the outline and the aims of this thesis.

In **Chapter 2** we studied the effect of the different governmental screening policies on the uptake of the FCT and PND over the periods from 2001 – 2003 (PNS on request), 2004 – 2006 (permission to offer the FCT to AMA women; women aged < 36 years informed on explicit request) and from 2007 – 2010 (introduction population screening) and we evaluated whether these trends were maternal age related.Analysis was done on data of the first- and second-trimester screening program for DS (N = 41.600) and on all invasive diagnostic tests (N = 10.795) performed from 2001 to 2010.

A significant increase was seen in the uptake of the FCT in the period 2001-2003 to 2007-2010. The uptake in women < 25 years, 26-30 years, 31-35 years, 36-40 years and 41-45 years increased with approximately 10%, 20%, 35%, 45% and 30% to 10.6%, 23.6%, 40.2%, 54.8% and 43.7% respectively in the period 2007-2010. The overall uptake of the FCT was 35.2% in 2010.There was a small decrease, however significant, in the number of PND for AMA (10%). A decrease was notable in women aged 36 - 38 years. Especially women aged ≥ 39 years opted for PND for AMA. Compared to the period 2001 – 2003 significantly more DS cases were detected with the FCT since 2004 in AMA women and since 2007 in women < 36 years. The choice for the FCT and PND for AMA seems dependent on the background risk. To accomplish a more effective screening policy the indication for PND for AMA should be abolished. The effect of screening will be visible at most if women only get access to PND after performing the FCT.

In **Chapter 3** we assessed the performance of the FCT in different maternal age groups over the period from 2004 until 2009. Of all the FCT performed (N = 26.274) 70.6% was done in women < 36 years of age. In this age group 43% of the DS cases were detected. For women aged < 36 years and for AMA women DR and FPR were 94.5% and 4.1%, 95.8% and 13.0% respectively. The balance between DR and FPR was even better in women < 36 years of age. Lowering the cut-off to 1:150 in women aged < 36 years and to 1:100 in AMA women resulted in an improved balance in DR and FPR (94.3% and 3.4% in women aged < 36 years and 94.2% and 6.7% in AMA women). This study strengthened the idea that the FCT is effective for women of all ages and that the routine offer of diagnostic testing to AMA women as screening policy for the detection of DS is not reasonable. Moreover reimbursement of the cost of the test should apply to all women

In **Chapter 4** the marker distribution of fβ-hCG and PAPP-A in singleton pregnancies conceived by assisted reproduction techniques (ART) was evaluated.
In a retrospective case control study (2004 – 2007) 203 IVF and 192 ICSI cases who performed the FCT were selected from a database of 14.645 cases (overall study group) and were compared to 1164 controls, matched for gestational age at serum sample date and maternal age. All ART cases were based on fresh embryo transfer with hormone treatment. Cases with adverse pregnancy outcome, like pregnancy induced hypertension, intrauterine growth restriction, intrauterine fetal death and preterm delivery (< 37 weeks), were excluded because of the association of these conditions with low PAPP-A levels. The data were log-transformed because of skewed distributions.
Compared to the matched controls significantly lower lnPAPP-A values were found in IVF (6.74 versus 7.08; *P* = 0.0001) and ICSI cases (6.59 versus 7.07; *P* = 0.0001) and lnfβ-hCG value was lower in the IVF group (3.75 versus 3.90; *P* = 0.005) and comparable in the ICSI group (3.87 versus 3.93; *P* = 0.27). Correction factors were computed for PAPP-A and fβ-hCG: IVF 1.42 and 1.17; ICSI 1.56 and 1.05 (not significant). The FPR in the IVF and ICSI group compared to the matched controls was higher (in IVF 10.3% versus 8.6% and in ICSI 10.9% versus 7.5%). In the overall age-biased (maternal age significantly lower compared to the ART cases and matched controls) study group the FPR was

6.8%. Adjustments of serum parameters values in ART cases in the risk analysis for DS are suggested.

In **Chapter 5**correction factors for fβ-hCG and PAPP-A in ART pregnancies (complicated and non-complicated) were applied and the effect on the FPR of the FCT was studied in comparison to an overall screening population. Data of 249 IVF and 250 ICSI cases were selected from an overall screening population in the period from 2008 – 2010 (N = 20.190). Only ART cases based on fresh embryo transfer with hormone treatment were included. Corrections (for the IVF cases 1.42 for PAPP-A and 1.17 for fβ-hCG and for the ICSI cases 1.56 for PAPP-A) were applied to the absolute serum concentrations.
Before correction mean PAPP-A MoM values were significantly lower (IVF 0.757 and ICSI 0.671 MoM) and after correction comparable (IVF 1.071 and ICSI 1.048 MoM) compared to the controls (1.004 MoM). Mean fβ-hCGMoM values were comparable before correction (IVF 1.054 and ICSI 1.051 MoMI) and significantly higher after correction (IVF 1.241 MoM) in comparison to the controls (1.062 MoM). After correction the odds ratio (OR) for receiving a false-positive result was not significantly different in IVF (OR 1.03; *P* = 0.248) and in ICSI pregnancies (OR 1.02; *P* = 0.448). Applying a correction for PAPP-A in ART pregnancies based on fresh-embryo transfer with hormone treatment reduces the FPR to the observed FPR in the overall screening population. The necessity of applying a correction for ART pregnancies based on fresh embryo transfer with hormone treatment is emphasized.

In **Chapter 6** we evaluated screening performance of the FCT with serum sampling at different gestational weeks and in different maternal age groups.
Data of the first-trimester screening program from 2005 - 2011 (N = 35.514) were used, including 145 DS cases and subdivided for the different gestational weeks (week 9, 10, 11, 12 and 13). Of the 145 DS cases 135 were detected. In the DS cases the accuracy of PAPP-A values early in pregnancy outweighed the accuracy of fβ-hCG later in gestation. No significant differences were found between the detection rates with serum taken at different gestational weeks. Compared to the FPR in week 9 (6%), the FPR in week 10 was comparable (6.5%) and the FPR in week 11(7.2%), 12 (7.4%) and 13 (8.5%) was significantly higher. When subdivided for different maternal age groups, a significantly increased likelihood of receiving a false-positive result was found when serum was taken from week 11 (OR 1.32; *P* = 0.008) for AMA women and from week 12 (OR 1.28; *P* = 0.04) for women aged < 36 years. The increase in FPR in AMA women in week 13 (OR 1.77, 95% CI 1.4 – 2.25) was significantly higher than in women aged < 36 years (OR 1.33, 95% CI 1.02 – 1.74). The importance of early serum sampling needs to be mentioned in the pre-test counselling. Early serum sampling should be implemented as a screening policy.

In **Chapter 7** screening performance for DS with the absolute risk (AR) method was assessed in comparison to the FCT. With the AR method maternal age risk is excluded from the risk algorithm.

Retrospectively we analyzed 32.448 data on the FCT. AR was defined as final risk divided by maternal age risk. The best cut-off value was determined for the AR method and then screening performance was compared between the AR method and the FCT for different maternal age groups.
The Receiver Operating Characteristics (ROC) of both methods were not significantly different. The best cut-off value for the AR method was 1.5. DR was comparable between both methods in all age groups. Two extra DS cases were detected in women aged < 30 years with the AR method, but 3 cases were missed in AMA women. Using the AR method the overall FPR was significantly lower (4.8% versus 6.8%; *P*< 0.001).) with a decrease in FPR in women aged 36-40 years (4.6% versus 12.4%), in women aged 41-45 years (3.9 versus 29.9%), and with an increase in FPR in women aged ≤ 25 years (6% versus 2.9%). A lower FPR would result in a reduction of the number of PND for increased risk. The AR method will take away the differences in screening performance at different maternal ages. This might lead to a higher uptake of first-trimester screening resulting in a more efficient screening policy.

Chapter 8 describes a general discussion, recommendations and future perspectives. In conclusion, the effect of implementation of PNS is limited with a low uptake of the FCT and a minimal decrease in PND for AMA. To accomplish a more effective first-trimester screening policy several adjustments of the current screening policies are necessary to improve the uptake of the FCT and improve screening performance. Barriers in access to screening and in the decision-making process should be removed. Everyone should be able to make an autonomous decision whether or not to participate in PNS without being hindered by financial burden.So first of all reimbursement of cost should apply to all women. Furthermore information provision should be related to educational level, adjusted for the different ethnic minorities and be provided timely, preferably in a preconceptional care consultation. Moreover the information should be more individually oriented and women should know that PND for AMA is an inappropriate screening tool for the detection of chromosomal abnormalities. The FCT should be the first step in prenatal testing because it provides a good risk assessment for women of all ages and thus PND for AMA should be abolished. Improving the information offer to pregnant women requires more education of the counsellors. Ongoing monitoring of the procedure for providing information about PNS is necessary, in order to assess whether the objective of informed decision-making is fulfilled.
For the current screening policy, the two-step screening approach with serum sampling before 11 weeks of gestation should be implemented. More studies are needed to study the effect of exclusion of maternal age risk from the risk assessment. When similar results are found, introduction of the absolute risk method should be considered, regarding the low FPR for women of all ages and the better positive predictive value. Finally it would be advisable to apply different cut-off values for women < 36 years of age and AMA women, for both the FCT as the absolute risk method.
In the near future non-invasive prenatal testing (NIPT) by analysis of cell-free fetal DNA in maternal plasma for the detection of DS, trisomy 18 and trisomy 13 will be available in our country. Before NIPT will be implemented as part of standard obstetric care, the most effective screening policy is to maintain the FCT as the first step in PNS. Women with an increased risk of having a baby with trisomy

21, 18 or trisomy 13 will be offered NIPT. Gradually the cut-off for increased risk can be expanded until NIPT will be available for all women. However in women with multiple gestations the accuracy of NIPT is not (yet) sufficiently validated. Also relevant will be the results of a study assessing pregnant women's decision making with regard to NIPT, their attitudes and opinions on NIPT. With the implementation of NIPT, the FCT will disappear as a screening tool for chromosomal abnormalities. As a result shifting towards screening in the first-trimester of pregnancy with focus on anomalies and pregnancy outcome is to be expected.