NIPT : non-invasive prenatal testing : towards implementation in the Netherlands

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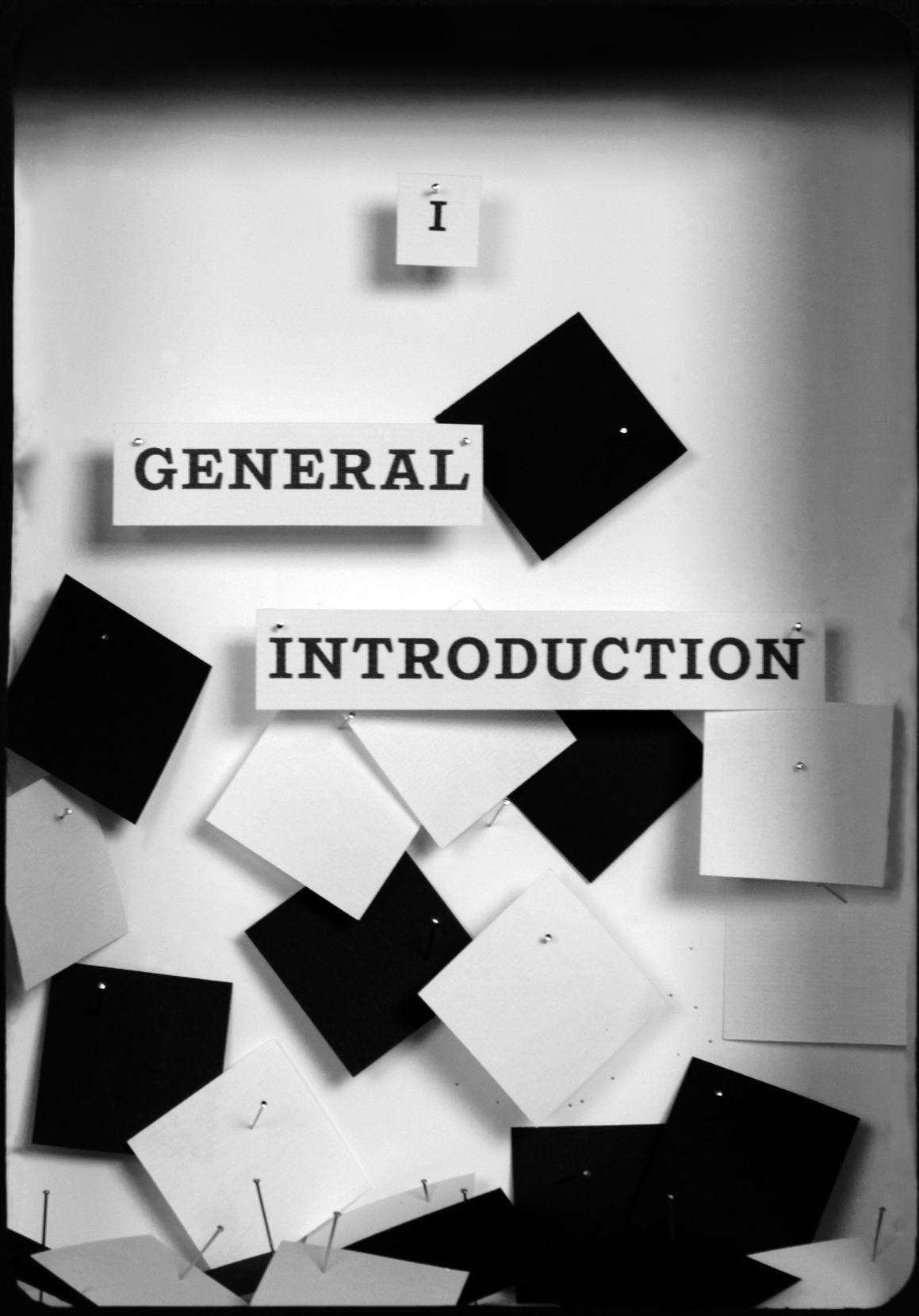
I
GENERAL
INTRODUCTION

The aim of providing prenatal screening for chromosomal conditions is to enable reproductive choice with respect to carrying to term or terminating the pregnancy of a child with a serious disorder or disability.[1] To elect for prenatal screening or diagnosis is a patient's choice.

CURRENT PRENATAL SCREENING PROGRAM FOR FETAL CHROMOSOMAL ANOMALIES

The most common chromosomal abnormality in live born children is Down syndrome (trisomy 21 (T21)). The prevalence of Down syndrome in the Netherlands is estimated to be 1:500. The risk for T21 is age related, the older a women is during pregnancy the higher the risk of an affected child.
In most western countries, pregnant women are offered prenatal screening for T21. In figure 1 the history of prenatal screening in the Netherlands until 2013 is depicted. Before 2007, an invasive procedure was offered to women of 36 years or older, based on the age-related risk. Age alone yet is a poor predicator for T21. Around 1% of the results were positive for T21, but due to the procedure, 0,5-1% of (most often healthy) pregnancies were lost. For this reason in 2007 a prenatal screening program was launched to predict the risk of T21 more precisely. In the first trimester, women are counselled about the option of the so-called first trimester combined test (FCT). The FCT is an individualised risk-calculation to estimate the chance of carrying a fetus with T21. The test algorithm consists of maternal age, maternal serum markers and nuchal translucency measurement and can be performed between 11-14 weeks of gestation (figure 2).[2] The nuchal translucency is a fluid accumulation behind the fetal neck, and is associated with fetal trisomy, and many other anomalies such as heart defects. The accuracy of the measurement depends on the experience and precision of the sonographer. In addition, screening on trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome) is offered using the same test, with an adapted algorithm since 2010.
The prevalence of trisomy 13 and 18 is lower, however these syndromes are often lethal. Together with the introduction of the FCT, the 20 week-anomaly scan was introduced to screen for neural tube defects and other structural abnormalities.

The introduction of the FCT resulted in a significant reduction in invasive procedures and was considered a big step forward. The accuracy depends partly on the quality of the ultrasound resulting in a false negative rate of 10–25% in clinical practice.[3-7] In case of a false negative result women are falsely reassured after the FCT, though confronted with a child with T21 after birth. If a woman decides to choose for the screening by FCT in order to have the possibility of terminating an affected pregnancy, a false negative result is clearly an unwanted outcome.
The false positive rate of FCT is a choice that can be made using the test characteristics and the cut-off between a high and a normal risk (in the Netherlands 1 in 200), and is most often set at 5%. Therefore, 1 in 20 women will be referred for an invasive procedure, while >90% of them do not carry an affected child.
Another serious limitation of FCT is its restricted time-window of 11–14 weeks gestation. Women who are late for their first visit, for any reason, are not able to elect for the FCT.

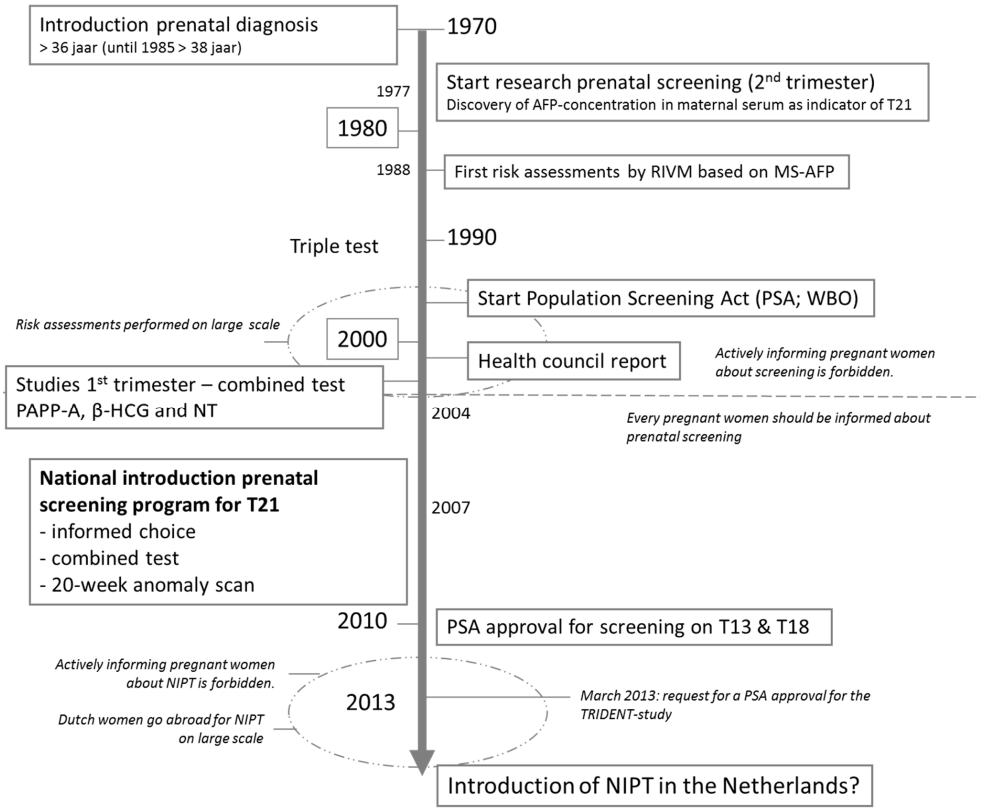

Figure 1. *History of prenatal screening in the Netherlands until 2013*

Invasive procedures

If a woman receives a FCT result with a high risk for trisomy 21,18 and 13, she is offered invasive testing using chorionic villus sampling (CVS) or amniocentesis. CVS is usually performed at 11 to 14 weeks of gestation either transabdominally or transcervically. Amniocentesis is the most commonly used invasive procedure and is usually performed from 15 weeks of gestation onward. Both the chorion villi as the amniotic fluid cells are investigated, mostly by rapid aneuploidy detection (RAD), short –and long term culture or a microarray. RAD using QF-PCR results in a quick result (2-4 days), detecting only the most common chromosomal abnormalities. Microarray is an extensive investigation, mostly applied only in case of an ultrasound abnormality after a normal QF-PCR or short-term culture.

These invasive tests are highly accurate and are associated with an iatrogenic miscarriage rate around 0.5-1%.[8,9] The CVS has a very high accuracy but the amniocentesis performs better. The accuracy of CVS is described to be 99.7% with a very small risk of failure due

to maternal cell contamination, clinical significance of mosaic confined to the placenta or laboratory failure.[10] In these cases resampling by amniocentesis is necessary. The accuracy of an amniocentesis is almost 100%. However, the actual procedure-related miscarriage rate remains a debate, as some obstetricians believe it is lower than 0.5-1%. Tabor et al. studied the fetal loss rate after an invasive procedure during an 11-year period in Denmark describing miscarriage rates of 1.4% (95% CI,1.3–1.5) after amniocentesis and 1.9% (95% CI,1.7–2.0) after CVS.[9] Another result of their study was that the number of procedures a department performed had a significant effect on the risk of miscarriage.[11,12] Wijnberger et al. observed this too in an earlier study where the learning curve for CVS was studied. They concluded that the operator experience influences the safety and success of the procedures.[13]
As described above, the majority of invasive tests (>90%) are carried out in pregnancies with a healthy fetus. However, women fear the invasive procedure, and have an anxious period waiting for the result. Although most of these women are reassured by a favourable result, the situation of strong anxiety is described to influence the pregnancy and the postpartum period negatively.[14] According to unpublished numbers of the National Institute for Public Health and the Environment (RIVM) only half of the women in the Netherlands with a high-risk assessment after FCT elect for an invasive prenatal diagnostic test. The reason for refraining from an invasive procedure after a high-risk assessment is unknown. It could be fear of losing the child after an invasive procedure, or fear for pain or needles, or depending on the actual FCT result, the feeling that the true risk is not really high.

Age related reimbursement

In the Netherlands, for most medical costs a fully covered health care insurance system provides equal health care for every citizen. Insurance companies therefore reimburse the 20-week anomaly scan for everyone. The government decided, however, that FCT for women <36 years was not to be included in the national insurance system. The costs of the FCT (2013: €154) for women ≥36 years are reimbursed. In case of a positive high-risk assessment after FCT further specialist counselling and invasive procedures are reimbursed.
Around 25% of the Dutch pregnant population decides to have a FCT performed. In comparison to other European countries this is a low uptake. Different reasons could account for this, like no desire to know whether the fetus has T21, characteristics of the test, the costs, the counselling, or not willing to take the risk of an invasive procedure following a positive result.

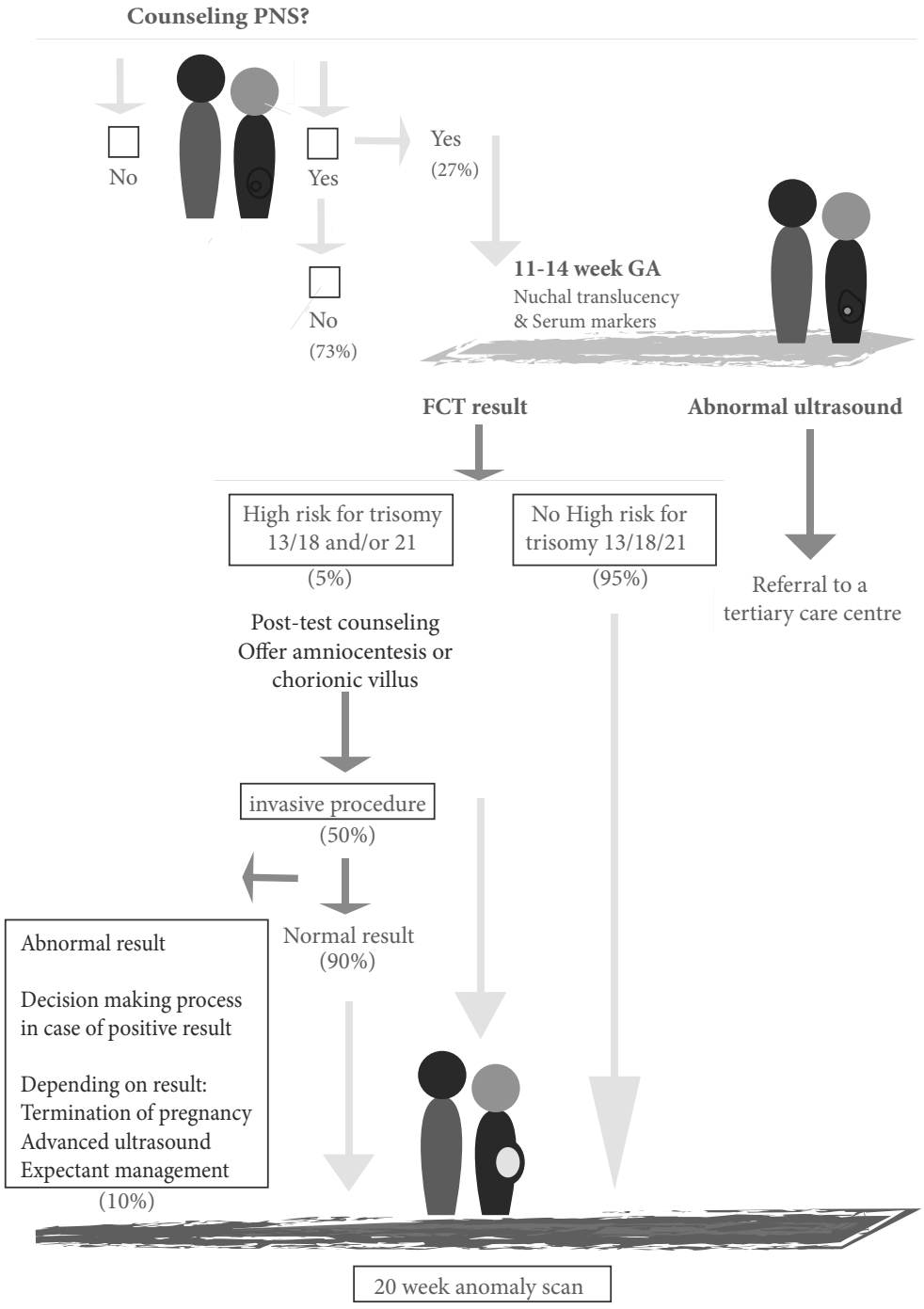

Figure 2. *Current first trimester screening program (estimated percentages)*

NON-INVASIVE PRENATAL TESTING (NIPT)

Background

Although the introduction of the prenatal screening program in 2007 was a big step forward, the current system has many disadvantages. Mainly because of the procedure-related miscarriage, other safer options for prenatal screening and diagnosis have been explored. First, years of investigation were done on fetal cells in the maternal circulation, but this was not successful.[15,16,17] Fetal cells can be detected in the maternal blood years after the pregnancy, even after miscarriages and for this reason are not reliable to evaluate chromosomal abnormalities of a specific pregnancy. In 1997, Dennis Lo et al. developed innovative methods for the analysis of fetal cell-free nucleic acids in maternal plasma and serum.[18] At first NIPT for fetal sex determination and rhesus D, technically easier than testing for fetal trisomy, have been investigated.[19] With the subsequent development of real-time quantitative PCR two proof-of-principle publications were published in 2008 using massively parallel shotgun sequencing (MPSS) to detect trace amounts of extra copies of chromosome 21 in the plasma of pregnant women carrying fetuses with T21.[20,21] The identification of T21 is more complicated because there are no unique fetal gene sequences, in contrast to a male fetus or a fetus with a D-gene in an R-D negative mother.[22]

The arrival of the MPSS or 'next generation' sequencing techniques has opened an era of many new options. A clinically applicable technology was developed for non-invasive testing of fetal chromosomal anomalies, using cell-free DNA (cfDNA) fragments of the placenta in maternal plasma. The development of NIPT for clinical use has been driven by several commercial laboratories in the United States and China. They invested many millions of dollars into this project over the last 5-10 years, because they believed that there would be a great demand, thus a market, for a safe and accurate fetal trisomy test. This seems indeed to be correct; in the first 2 years of the availability of their MaterniT21 test, the first company to launch NIPT (Sequenom Laboratories) already performed more than 200,000 tests worldwide.

Technique

In MPSS, the total amount of cell-free DNA fragments, consisting of a 'fetal fraction' thought to be mainly derived from the placenta and the much larger maternal fraction is sequenced. The relative amounts of plasma DNA molecules derived from the various chromosomes are analyzed. Trisomy 21 is caused by the presence of a third copy of chromosome 21. So in case of a trisomy 21 fetus an increased number of sequences are derived from chromosome 21. The maternal genome is mostly euploid, so abnormalities in the proportions originate from the fetal genome. The MPSS method as described above is currently most widely used. A directed cfDNA method, using digital analysis of selected regions (DANSR), combined

with an analysis algorithm, the fetal-fraction optimized risk of trisomy evaluation (FORTE) has been shown to have similar accuracy.[23-26] The third method is the single nucleotide polymorphisms (SNPs) approach, where polymorphic loci are selectively sequenced on the different chromosomes. When measured between 10 and 20 gestational weeks, the average fetal fraction in the maternal plasma is 10% to 15% but can range from under 3% to over 30%. Screening performance is better with increasing fetal fraction. The strongest factor associated with low fetal fraction is high maternal weight.[27]

Performance

Both the sensitivity and specificity of NIPT for fetal T21 exceed 99%.[28] The published studies were generally performed in populations with a known high-risk for trisomies. In most studies archived samples were used, analyzed in batches, with a selected, known to be normal control group.

In 2011, NIPT for fetal trisomy was introduced in clinical practice in the USA, China, and Hong Kong. After introduction of the test, more studies have been performed.[28] The performance of NIPT, for high-risk populations in prospective studies is very accurate for T21 but a higher false positive rate and false negative rate is reported especially for trisomy 13 (T13).
Explanations for false positive NIPT results include technical reasons, the presence of confined placental mosaïcism, or a lost, perhaps unrecognized, co-twin which may provide an increased amount of DNA fragments in maternal plasma. In such cases the test itself can be considered true positive on a cfDNA level, however, the fetus may have a normal chromosome configuration. Some researchers suggest calling such results 'discordant' instead of false positive.

INFORMED DECISION-MAKING

In the light of all these exciting technological opportunities the importance of the social and ethical considerations should not be underestimated.
In the Netherlands the antenatal screening program is designed to provide every pregnant woman the necessary information to make an informed choice about whether or not to request FCT. Women should be able to make an autonomous decision. Multiple factors influence pregnant women in their decision to accept or decline prenatal screening.
Parity, fertility history, family history for chromosomal anomalies, education level, ethnicity and religion are acknowledged to attribute in women's choices for prenatal screening.[30] Until now no studies were performed on the influence of personal costs on the decision-making process. The main reasons to request the test are reassurance and the desire to have knowledge

about the health of the fetus.[31,32] The decision to decline FCT seems to be connected with the woman's view on termination of pregnancy (TOP).[31,32] At present, the vast majority of women confronted with a confirmed diagnosis of fetal trisomy request TOP. In the Netherlands, 93% of women receiving the diagnosis fetal T21 terminate the pregnancy (according to the 2010 annual report on prenatal diagnosis). Some women receive the diagnosis of fetal T21 after 24 weeks of gestation; in this situation it is not legally possible to terminate pregnancy in the Netherlands.

With NIPT, decision-making in prenatal screening is likely to change. Ethical debates revolve around the issue of a possible consequence of this increased testing rate: 'Will the world be without children with Down syndrome in a few years?' There is also concern by some, often quoted in the media, that increased testing with likely reduced numbers of live-born children with T21 may lead to less acceptation of people with T21 in society, or a change towards blaming their parents for their birth.

DUTCH SITUATION

Towards NIPT in the Netherlands

Although other studies were published on NIPT, the Dutch media suddenly broadly covered the subject of NIPT in March 2011, after the publication of Papageorgiou et al.[33] In the same month the first steps towards a national consortium were made. All stakeholders including all Dutch academic centres participated. Several meetings followed to design a national study (the so-called Non-Invasief TRisomie Onderzoek (NITRO)-study) to investigate the feasibility and real time diagnostic accuracy of NIPT, a head-to-head comparison with the FCT in Dutch laboratories. A website was designed as a platform on NIPT for all participating stakeholders and for patients (www.niptconsortium.nl). On the website there is a part secured by a password for participating stakeholders. In 2011 and 2012, meetings with the Ministry of Health, Health Council and Health Insurance Companies were organized to open the dialog about the implementation of NIPT in the Netherlands including a request for Population Screening Act approval. There was, and still is, considerable discussion among professionals as to whether such an approval would be needed, since the proposed application of NIPT would first be only as an alternative for the diagnostic tests in screen-positive women, amniocentesis and CVS.

Population Screening Act

Prenatal screening for untreatable disorders can only be performed if there is a permission of the Minister of Welfare, Health and Sports (VWS) according to the Population Screening Act (PSA; Wet op Bevolkingsonderzoek - 1996).[34,35] The Population Screening Act provides for a permit system for population screening involving the use of ionising radiation, concerning

cancer or concerning serious diseases or abnormalities for which no treatment is possible (www.gr.nl). Ministerial approval is needed for every adjustment on a screening program, so is the case for the implementation of NIPT. The Health Council has an advisory function.

AIM OF THE THESIS

Because of the good test characteristics NIPT will undoubtedly find a place in our healthcare system. The aim of this thesis is to explore and gain more insight regarding the future implementation of NIPT in the Netherlands. Careful preparation for the implementation is essential. Many possible consequences of the implementation of NIPT for pregnant women are unknown.
Research questions were:

- What is the available evidence published about the performance of NIPT for fetal trisomy?
- Is it feasible to send maternal blood samples to laboratories in the United States, and what is the diagnostic accuracy of NIPT using this route?
- Non-invasive prenatal testing for T13; does it do more harm than good?
- Will there be changing attitudes towards termination of pregnancy with the implementation of NIPT?
- What do pregnant women want with the introduction of NIPT?
- What is the influence of personal costs on the decision making process for prenatal screening?
- What is the expected uptake of NIPT?
- What is the attitude of Dutch midwives on the current screening program and on NIPT?
- The Population Screening Act protects the population for potential harm, but also brings a moral dilemma to caregivers. What to do if a test is superior for your patients, but you are not allowed to offer the test?
- And what are the options for Dutch women to have testing by NIPT done outside the Netherlands?
- The next step – an implementation program – what are the most important issues that need to be solved?

OUTLINE OF THE THESIS

Part I. General introduction
Part II. Performance

- Detecting fetal trisomy using cell-free DNA in maternal plasma has been challenging, but after decades of research it is now feasible, and the diagnostic accuracy appears high. We aimed to systematically review the published literature on accuracy of NIPT for the prediction of T21 (1997 – May 2011) using the QUADAS guidelines. This review is described in chapter 1.
- NIPT was not available in the Netherlands at the time of writing the thesis. In 2011/beginning 2012 pregnant women wanting NIPT travelled to the United States. Shipping blood samples across the ocean instead of pregnant women flying to the United States seemed a more feasible way. For this reason the primary aim of the EU-NITE study, discussed in chapter 2 was to evaluate the performance of a directed non-invasive prenatal testing method of cell-free DNA analysis for fetal T21 by shipping whole blood samples from Europe to a laboratory in the United States.
- Chapter 3 discusses some of the potential disadvantages of NIPT. The diagnostic accuracy of NIPT for T13 is reported to be lower. Screening for a lethal disease such as T13, with false positives leading to risky invasive procedures in healthy pregnancies, may do more harm than good.

Part III. Decision Making

- Currently 93% of the women who receive a positive result following an invasive procedure elect for TOP. With the elimination of the risk of an iatrogenic miscarriage, decision-making might change. In chapter 4 we sought to evaluate whether and how the assumed increased rate of detection with the introduction of NIPT would influence the rate of TOP for affected pregnancies.
- In chapter 5 two questions are evaluated. Currently the uptake of the FCT is low, compared to other countries. Earlier studies concluded that the test characteristics of the FCT and the iatrogenic miscarriage risk negatively influence the choice for electing FCT and invasive procedures. If a new test is implemented with better test characteristics the uptake will likely change. We sought to evaluate the attitude of pregnant women towards the future implementation of NIPT. Secondly we sought to evaluate the price that women would be willing to pay for NIPT, which may reflect how women value the risk-free NIPT.
- In Chapter 6 the influence of personal costs is discussed in the decision to undergo FCT. A study was performed comparing the number of women opting for FCT during a period of time where the test was fully reimbursed, with a more recent time-period where women younger than 36 years had to pay for the FCT themselves.

Part IV. Towards NIPT in the Netherlands

- In the Netherlands most pregnant women receive care by independent primary care midwives, including the counseling for the FCT. Until now it was not known what the attitude of primary care midwives is towards the current prenatal screening system and towards NIPT. The aim of the study described in chapter 7 was to investigate the attitude of primary care midwives towards the current system and towards NIPT.
- In 2013, offering NIPT was still forbidden in the Netherlands, since such a change in the national government-approved prenatal screening program requires a new version of the Population Screening Act license. Increasingly, pregnant women became aware of the option to have NIPT performed across the border, in Belgium and Germany. The Dutch NIPT Consortium has requested a license from the Minister of Health, to perform a prospective evaluation project of NIPT in high-risk pregnancies. In chapter 8, we discuss the situation at the time of writing this thesis concerning NIPT, ethical and legal considerations and advise for obstetric care professionals confronted with either requests from patients or their own desires to offer NIPT as an alternative to invasive testing, while awaiting formal permission to incorporate this test into clinical practice.

Part V. Opinion

- In chapter 9 we debated an opinion by Benn et al., published in Ultrasound in Obstetrics & Gynecology with the title 'Non-invasive prenatal diagnosis for Down Syndrome: the paradigm will shift, but slowly'.

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