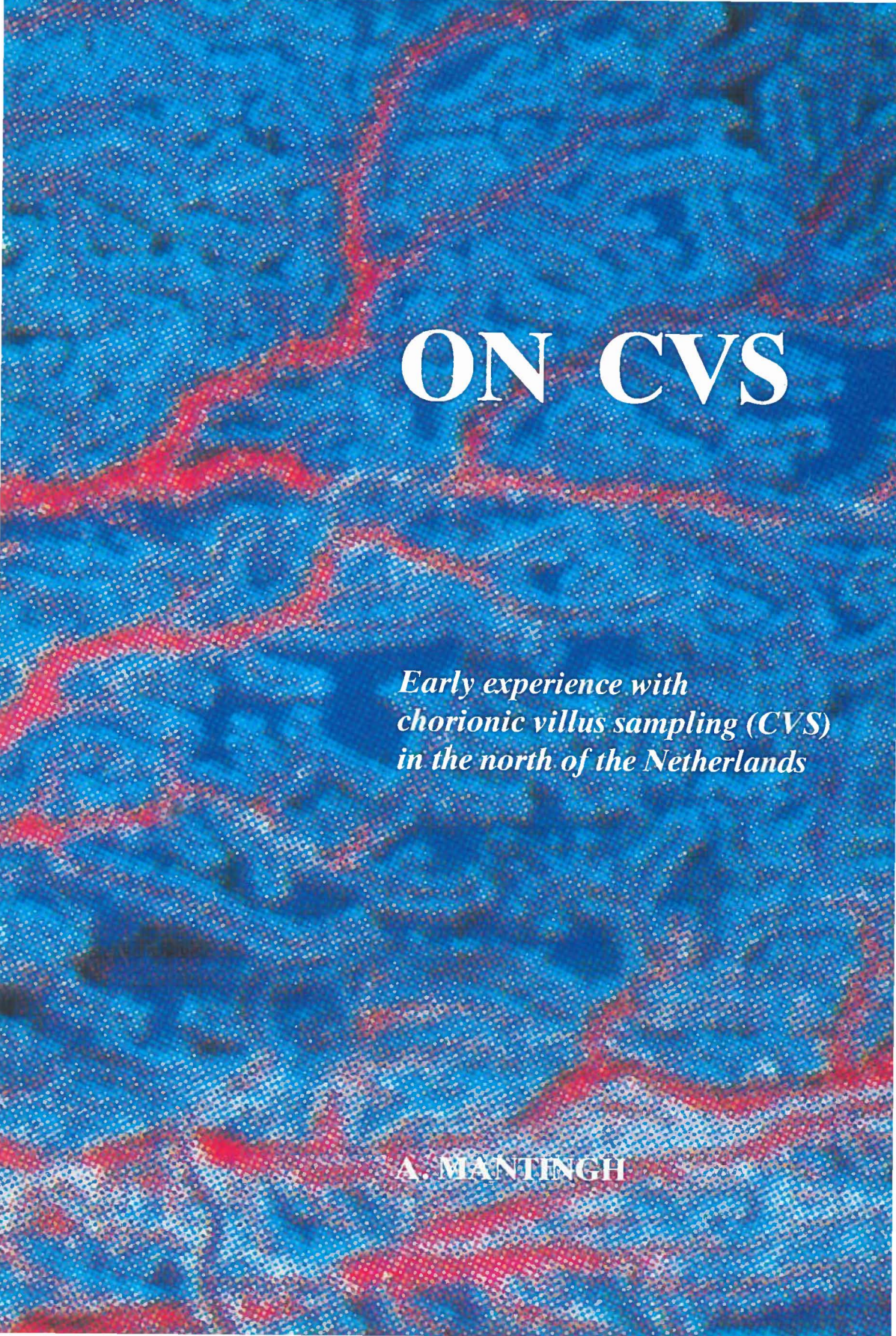

ON CVS
Early experience with chorionic villus sampling (CVS) in the north of the Netherlands
A. MANTINGH

ON CVS

Early experience with chorionic villus sampling (CVS) in the north of the Netherlands

The publication of this thesis was supported by the 'Stichting voor Erfelijkheidsvoorlichting (Foundation for Genetic Counselling), Groningen, the Netherlands.

ISBN 90.6148.997.0

Design and printing: Knoop Haren Gn
P.O. Box 16, 9750 AA Haren Gn, the Netherlands.

Stellingen behorende bij het proefschrift van

ALBERT MANTINGH

ON CVS

Early experience with
chorionic villus sampling (CVS)
in the north of the Netherlands

Groningen, 21 december 1988

1. Ook een te laag maternaal serum alpha-foetoproteïne in de 16e week van de zwangerschap moet aanleiding zijn tot vervolgonderzoek en dient aan de officiële indicatielijst voor antenatale diagnostiek te worden toegevoegd.

2. De afschaffing in 1985 van het chromosomenonderzoek van vruchtwater, afgenomen voor het onderzoek op neurale buisdefecten vanwege een 2e of 3e graads familiaire belasting, moet ongedaan worden gemaakt.

3. Het gekrakeel naar aanleiding van het recente rapport van de Gezondheidsraad 'Neuraalbuisdefecten' maakte vooral duidelijk dat wat de zwangeren er zelf van vinden volstrekt onbelangrijk gevonden wordt.

4. Een arts die geen ongevraagde informatie geeft over routine antenatale diagnostiek handelt niet onjuist.

5. Het is nog maar de vraag of ouders die na antenatale diagnostiek ook het geslacht willen weten hun kind daarmee een dienst bewijzen.

6. Het verwerpen van een abortus provocatus na antenatale diagnostiek vanwege 'ongewenst geslacht' is onverenigbaar met 'baas in eigen buik'.

7. Er is geen plaats voor immunotherapie in de behandeling van recidiverende abortus.

8. Zolang 'Primary Health Care' wordt beschouwd als 'second-rate, poor man's medicine' is het WHO streven naar 'Health for All by the Year 2000' tot mislukken gedoemd.

9. De positieve kant van medewerking van een Nederlandse arts in vreemde overheidsdienst aan 'medical fitness' onderzoek bij lijfstraffen is dat het de 'non-fits' in bescherming neemt.

10. De onmacht van de patiënt maakt de zogenaamde 'macht van de dokter', niet andersom.

11. Boycots spelen 'Apartheid' in de kaart.

12. In een land waar alles moet kunnen en niets hoeft geldt de wet van behoud van ellende.

13. De nieuwbouw in het AZG is een voorbeeld van desoriënterende architectuur.

14. In de discussie over genetische manipulatie vraag je je vaak af wie er eigenlijk wordt gemanipuleerd.

15. De hardste schijf zit tussen je oren.

16. Mileage and marriage *do* mix.

17. Ook dit proefschrift werd geboren uit turven, jenever en achterdocht.

RIJKSUNIVERSITEIT GRONINGEN

ON CVS

Early experience with chorionic villus sampling (CVS) in the north of the Netherlands

PROEFSCHRIFT

ter verkrijging van het doctoraat
in de Geneeskunde
aan de Rijksuniversiteit Groningen
op gezag van de Rector Magnificus Dr. L.J. Engels
in het openbaar te verdedigen op woensdag 21 december 1988
des namiddags te 1.15 uur precies

door

Albert Mantingh

geboren te Stadskanaal

PROMOTOR: Prof.Dr. H.J. Huisjes

PROMOTIECOMMISSIE:

Prof.Dr. G.J.P.A. Anders
Prof.Dr. J. Bennebroek Gravenhorst
Prof.Dr. J.D. Elema

This thesis was prepared at the Department of Obstetrics and Gynaecology, University Hospital, Groningen, the Netherlands.

'Is this song so different?
Am I doing it all again?
It may have been done before
But then, music's an open door…'

'The Who'

ACKNOWLEDGEMENT

The help received from patients, colleagues, friends and family was half the fun of preparing this thesis. Thank you!

CONTENTS

1 SUMMARY AND ABBREVIATIONS

1.1 Summary

During the first 2 years of our chorionic villus sampling (CVS) service (from November 1984 to October 1986 inclusive), 427 women applied for prenatal diagnosis early enough in pregnancy to be considered suitable candidates for CVS.

Of these women, 216 actually did undergo CVS. The other 211 did not, for various reasons.

The response rate of women for CVS and amniocentesis (indication maternal age $\geqslant$36 years) in the north of the Netherlands was equal to the Dutch national average (30%), although the differences between the respective provinces Groningen, Friesland and Drenthe were considerable.

The referral pattern for antenatal diagnosis shifted from second to first level health workers. (Chapters 2 and 3)

Sampling was performed transcervically under ultrasound guidance at around 10 weeks gestational age. (Chapter 4)

CVS failed in 15 women (6.9%): no villi or unsuitable ones were obtained in 12 women (sampling failure 5.6%)[1], while karyotyping was successful in all but 3 specimens (laboratory failure 1.5%). Results were abnormal in 10 women (5.0%), i.e. trisomy 21 (3x), mosaicism (3x), triploidy (2x), 'deletion-16' (1x), polyploidy (1x). True and false positives and their implications are described.

Ten pregnancies intended to continue to term, ended before the 28th week (4.8%): in week 12, 13, 16, 17(3x), 18, 20, 22 and 26, the latter infant survived.

Slight vaginal bleeding for a few days after CVS was the most common complaint.

All pregnancies intended to continue to term, had ended by the time of writing this thesis, the last one on 13 June 1987. (section 5.1)

The fact that we were unable to inform the women in our study group of the precise abortion risk, was their main reason to prefer amniocentesis over CVS. This, together with spontaneous abortion occurring before the test, was the main determinant of the drop-out group. (section 5.2)

Alpha-fetoprotein values in maternal serum (MSAFP) before and after sampling and at 16 weeks gestation, indicated considerable, but transient feto-maternal haemorrhage in the majority of cases. Also, low first trimester MSAFP concentrations were strongly associated with fetal trisomy 21. (section 6.1)

[1] One from Arnhem included (section 5.1).

Fetal motility patterns just before CVS hardly differed from those directly after CVS, even when CVS had been quite traumatic. (section 6.2)

The main CVS procedural characteristics did not relate to particular problems in pregnancy, nor to specific placental pathology. (section 6.3)

Some psychosocial aspects of decision making between CVS and amniocentesis were remarkable. The women tended to underestimate the problems associated with the long wait for the amniocentesis result and the possibility of a termination of pregnancy relatively late in pregnancy. Moreover, some women would definitely have preferred being informed about the results in writing rather than by telephone. (section 6.4)

CVS proved to be a valuable addition to amniocentesis, though with limitations. Its merits make it the method of choice for many women, despite important issues not yet settled and presently existing uncertainties. Some problems seem to be smaller than initially thought (especially abortion risk), but others larger (discordant results).

The true picture of how CVS relates to amniocentesis should be obtained by comparing both these methods of antenatal diagnosis in a randomized trial. In the Netherlands the opportunity to do so has passed.

1.2 List of abbreviations

List of generally used abbreviations in alphabetical order:

AAP = Abortus arte provocatus
AC = Amniocentesis
AFP = Alpha-fetoprotein
BPD = Biparietal diameter
CRL = Crown-rump length
CVS = Chorionic villus sampling
NTD = Neural tube defect
TOP = Termination of pregnancy
Tri.21 = Trisomy 21 (= Down' syndrome)
US = Ultrasound

Abbreviations that are not used throughout, but in particular sections only, are given there.

2 INTRODUCTION AND AIM OF THE STUDY

2.1 Introduction

The following sections introduce the subject of chorionic villus sampling (CVS) and analysis as a diagnostic tool for detecting congenital abnormalities antenatally:

2.1.1 Semi-schematic time-table of historical milestones in antenatal diagnosis technologies in general (left hand column), coupled with essential prior developments (right hand column).

2.1.2 Semi-schematic time-table of historical milestones of CVS with essential preliminaries.

2.1.3 Situation world-wide since the clinical introduction of CVS.

2.1.4 Situation in the Netherlands since the introduction of CVS.

2.1.5 Situation in the northern provinces of the Netherlands.

2.1.6 Future prospects.

2.1.1 Historical time-table antenatal diagnosis technologies in general	*Essential preliminaries*
1950	
1950: Amniocentesis for haemolytic disease in the newborn (blood pigments) (Bevis 1950, 1952, 1956).	
1955: Amniocentesis for fetal sex determination (Barr bodies) (Serr et al 1955, Fuchs and Riis 1956, Shettles 1956).	
	1956: Establishment of the number of chromosomes in man as 46 (Ford and Hamerton 1956, Tjio and Levan 1956).
	1958: Introduction of ultrasound in obstetrics (Donald et al 1958).
	1959: Demonstration of chromosomal basis of Down's syndrome (Lejeune et al 1959).
1960	
	1966: Karyotyping of cultured amniotic fluid cells (Steele and Breg 1966, Thiede et al 1966).
1967: Amniocentesis for chromosomal disorders (Jacobson and Barter 1967).	
1968: Amniocentesis for inborn errors of metabolism (Nadler 1968).	

1970	
1971: Prenatal diagnosis of fetal sex simplified (Arendzen and Huisjes 1971).	
1972: Amniocentesis for neural tube defects (alpha-fetoprotein) (Brock and Sutcliffe 1972).	1972: Karyotyping of uncultured cells (mice) (Evans et al 1972).
1972 Sonar scanning for neural tube defects (Campbell et al 1972).	
1973: Maternal serum alpha-fetoprotein measurement for neural tube defects (Brock et al 1973).	1973: Introduction of fetoscopy (Scrimgeour 1973).
1974: Fetal bloodsampling by fetoscopy for haemo-globinopathies (Hobbins and Mahoney 1974, Golbus et al 1976).	
1975: Chorionic villus sampling for fetal sex determination (Barr bodies) (Anshan 1975).	1975: Karyotyping of cultured chorionic villi (Yamamoto et al 1975).
1980	
1983: CVS for chromosomal disorders (Brambati and Simoni 1983).	1983: Karyotyping of uncultured chorionic villi (Simoni et al 1983).
1983: Ultrasound-guided fetal bloodsampling by cordo-centesis (Daffos et al 1983, Nicolaides et al 1985, Hobbins et al 1985).	
1987: First trimester amniocentesis (Ashmead et al 1987, Cordone et al 1987, Hanson et al 1987).	

2.1.2 Milestones in clinical chorionic villus sampling (CVS) technology.	*Essential, and partly 'experimental' preliminaries.*
1950	
	1958: Transcervical placental biopsy for hydatidiform mole (Acosta-Sison 1958).
1960	
	1966: Transabdominal placental biopsy for hydatidiform mole (Alvarez 1966).
	actual experimental take-off:
	1966 (Denmark): In 55 patients transcervical first trimester CVS under direct (hysteroscopic, 6 mm diam.) vision prior to elective abortion was performed as an alternative to late, second trimester amniocentesis in genetically high-risk pregnancies (X-linked, metabolic). Problems: unclear view, puncturing amniotic sac, aspiration of membranes instead of villi only. Subsequent culture of villi fairly successful though subsequent analysis remained difficult. It was thought that 50% of pregnancies intended to continue to term would not survive interference of this kind: a bad deal compared to amniocentesis; work was discontinued (Mohr 1986, Hahnemann and Mohr 1968, 1969, Hahnemann 1974).

1973 (Sweden): The above Hahnemann procedure was repeated with a modified (5 mm diam.) 'endocervicoscope' in pregnancies scheduled for elective abortion. Fewer aspiration-problems arose, instead 2 complications of gonococcal amnionitis. Major problems in analyzing cultured villi still encountered. Follow-up research not pursued (Kullander and Sandahl 1973).

1975 (China): 'Blind' transcervical CVS technique (metal cannula, 3 mm diam.) for fetal sex determination ('to help women who desire family planning') by examination of sex chromatin in villus material, followed by induced abortion if 'wrong' sex. Accuracy 94%; 4 spontaneous abortions (3-7 weeks after CVS) in 66 pregnancies intended to continue to term (6.1%) (Anshan 1975).

1975 (USA): Prenatal sex detection with endocervical smears by Y-body fluorescence (Rhine et al 1975).

1979 (USA): Cells obtained from normal saline uterine washouts (Rhine and Milunsky 1979) hardly suitable for fetal chromosome diagnosis (Goldberg et al 1980).

1980

1981 (UK): Successful gene mapping on chorionic villi (Williamson et al 1981), as well as culture and subsequent karyotyping of small amounts of chorionic villi (Niazi et al 1981).

1982/1983 (UK and France): First haemoglobinopathies diagnosed by DNA analysis of chorionic villi (Old et al 1982, Goossens et al 1983, Kaplan et al 1983, Horwell et al 1985).

1982 (USSR): Four different approaches in a mixed experimental setting. Visualization by either 'embryo-fetoscope' (1.7 mm diam.) or real-time ultrasound (and biopsy forceps 2 mm diam.) either prior to elective abortion or in planned high-risk (mostly X-linked) pregnancies for fetal sexing by chromatin examination. Some enzyme activity levels could also be determined. All 13 'unaffected' pregnancies continued normally. This was the first time ultrasound was used to visualize the CVS procedure (Kazy et al 1982).

big clinical take-off:

1983 (UK and Italy): Transcervical CVS with a small (1.5 mm diam.) plastic suction cannula with a malleable aluminium obturator. Insertion and aspiration under continuous ultrasound and immediate microscopic check of aspirate for presence of suitable villi, found to be the best approach (Ward et al 1983, Simoni et al 1983, Rodeck et al 1983).

1983 (Italy): Development of method for direct chromosome preparation of uncultured chorionic villi for quick karyotyping (Simoni et al 1983), based on earlier animal experiments (Evans et al 1972) and spontaneous abortion karyotyping (Yamamoto et al 1975).

1983 (UK): Poor results with 'blind' transcervical aspiration (Liu et al 1983).

1983 (Italy): First fetal trisomy 21 diagnosed within 5 hours after CVS by direct karyotyping (Brambati and Simoni 1983).

1983 (UK): Fetal sexing for Duchenne's muscular dystrophy (Williams et al 1983, Lilford et al 1983).

1983 (US): Diagnosis of Tay-Sachs disease by CVS (Pergament et al 1983, Grebner et al 1983).

1983 (Sweden): Good CVS results using hysteroscope instead of cannula (Gustavii et al 1983, 1984).

1984 (Netherlands): Determination of fetal blood group and Rhesus factor from chorionic villi fetal erythrocytes (Kanhai et al 1984, 1985).

1984 (Netherlands/France): Diagnosis of Hunter syndrome (Kleyer et al 1984), followed by several other metabolic diseases (Kleyer et al 1986, 1986, 1987).

1984 (Denmark): Transabdominal approach as successful as transcervical (Smidt-Jensen and Hahnemann 1984, 1985).

1984 (Hungary): Second trimester CVS as an alternative to cordocentesis (Szabó et al 1984).

1984: (UK): Diagnosis of Lesch-Nyhan's syndrome by CVS (Gibbs et al 1984).

1985 (France): Also good results using rigid forceps instead of malleable cannula (Dumez et al 1985).

1985 (Netherlands, UK, France, USA, Canada): Diagnosis and carrier detection of Duchenne muscular dystrophy by DNA analysis (Bakker et al 1985).

1985 (UK): Demonstration of fragile-X in chorionic villi (Kearny 1985).

2.1.3 Situation world-wide since the clinical introduction of chorionic villus sampling (CVS)

Brambati's first report of trisomy 21 diagnosed by CVS within a few hours after sampling caused quite a stir (Brambati and Simoni 1983). Coupled with Simoni's direct method for chromosomal analysis of uncultured villi, this formed a really attractive alternative for second trimester amniocentesis.

The scene was set and although the World Health Organization initially took the lead in setting up a CVS monitoring system to establish the safety and efficacy of the procedure, it has been more or less overtaken by Dr. Laird Jackson's CVS registry. (Jefferson Medical College, Philadelphia). 'The registry tabulates voluntary reports of CVS – world-wide' and now acts as an international forum as practically all centres report their CVS procedures and follow-up regularly and in a uniform way. From these reports an up-to-date 'Fetal Loss Registry' and a 'Cytogenetic Experience Registry' is construed and published in the 'CVS News-letter' every few months.

By the end of 1987 over 35,000 CVS cases had been registered, with a total fetal loss rate (< 28w) of between 3.5% and 7.4%, depending on the type of catheter used and on the type of approach (transcervical or transabdominal) (Jackson 1987/23).

In 1984, Dr. D.T.Y. Liu organized the first international CVS Symposium in Nottingham, thereafter held annually: in Birmingham 1985, Strasbourg 1986, Chicago 1987 and Athens 1988.

From the beginning, the main concern with CVS has been its safety. Viewed against the bulk of the procedures being conducted in a low-risk (1-2%) group of women, it is mandatory to establish the exact procedure-related risk. For

amniocentesis it is still regretted that its added risk has never been precisely established by means of a randomized controlled trial, putting amniocentesis against no amniocentesis. Therefore, it is necessary to approximate the procedure-related abortion risk and other obstetrical problems by collecting figures on all problems after amniocentesis and subtracting the number of problems thought to be 'natural background problems' in such a population (exact figures being unknown). However tight the controls in a controlled matched study are (e.g. The UK Medical Research Council, 1978), basic flaws will remain and yield conflicting results when compared with studies of a similar design (e.g. a UK fetal wastage rate of 1.5% directly attributed to amniocentesis but not confirmed in comparable studies in Canada and the USA (Report MRC 1978, Simpson et al 1976, and NICHD report 1976, respectively)). *'A most unsatisfactory state of affairs' says Brock (1982) and proceeds: 'taking all available evidence together, the practising obstetrician would probably be wise to quote a risk of fetal loss of the order of 4%, but also to emphasize to his patient that she has an approximately 3% risk of miscarriage even if he does not perform amniocentesis'*. The difficulty of the problem is quickly recognized. Compared with an average low-risk figure of 1% for fetal chromosomal abnormalities, a varying procedure-related risk of somewhere between 0.5% and 2% could make quite a difference to a particular patient as such a risk then ranges from half to almost twice as high compared with her risk of fetal chromosomal abnormality. In practice such a comparison is often made as the maternal age-related risk for chromosomal anomalies starts to become larger than the amniocentesis-related abortion risk (put at 0.5%) from 36 years onwards. On the other hand, I doubt whether the result of this sort of weighing is really decisive: the abortion risk and the risk of Down's syndrome are incomparable. Most candidates for antenatal diagnosis would not at all cherish the thought of being randomized, at least not here in the Netherlands, where they have usually made up their minds by the time they come for intake (see also Appendix 11.6).

Yet, the epidemiologically-orientated amongst us have seized the opportunity to put the new procedure of CVS to the test in a decent trial: again, not randomizing against no antenatal diagnosis at all, but against amniocentesis, therefore enabling at least the proper determination of the pros and cons of each method. The Perinatal Epidemiology Unit in Oxford took the initiative of organizing such a trial (Chalmers 1984). Despite convincing support from several quarters (e.g. Modell 1985, Editorial 1985), the effort met a mixed response and cooperation initially promised, often turned out to be so half-hearted as to be virtually non-existent (e.g. in the Netherlands, see section 2.1.4). Nevertheless, the trial was started, seems to be well under way by now and has even been joined by similar efforts elsewhere (Canada, Denmark, Finland).

Chalmers (1984) calculated the figures needed to demonstrate significant differences between CVS and amniocentesis for particular aspects e.g. estimation of overall fetal loss, as shown in Table 2.1.[1]

[1] Three outcome measures were actually proposed: 1. rate of overall fetal loss, 2. rate of spontaneous loss of chromosomally normal fetuses and 3. rate of important postnatal complications, including long-term problems during childhood.

Table 2.1 Total number of pregnancies required in each group CVS/ amniocentesis, if the difference is as specified (significance = 5%; power = 80%, Chalmers 1984).

		CVS percentage loss overall								
		2	3	4	5	6	7	8	9	10
	1	2316	767	423	284	211	166	136	115	99
	2		3821							
Amniocentesis	3			4200						
percentage	4				5670					
loss overall	5					8149	2210			
	6						*	2600		
	7							*	2784	
	8								*	2900
	9									*

* = > 10.000

This is long-range work and the reason that clinicians are half-hearted about this kind of exercise is not because they do not see the relevance of the questions posed, but because they are under the impression that they are in no dire need of the answers. Thus, the effort required to obtain them is not justified. Judging from their lukewarm response, they obviously think they can do pretty well without them. I.e., a broad approximation, say an abortion risk of between 2% and 4%, would suffice for practical purposes.

I hope to show that such an approximation based on a meticulous and above all, sceptical follow-up, can be given precisely enough as to satisfy the basic requirements of decent counselling. Admittedly, a second best approach, but the best given the circumstances.

2.1.4 Situation in the Netherlands since the clinical introduction of CVS (1984-1987)

The Dutch pioneers in Rotterdam (Jahoda et al 1984), having been initiated by the Milano team of Brambati c.s., in turn helped others to make a start (e.g. Groningen).

A 'working party' consisting of obstetricians and clinical geneticists occupied in the field of antenatal diagnosis started to convene on a regular basis. Initially the group strongly supported the Oxford multicentre trial and even expressed its intention to collaborate (in Groningen the project had already formally passed the Medical Ethics Committee). This initiative stumbled some while later and never actually got off the ground properly (see Appendix 11.6). The Dutch contribution in general remained poor throughout, with only a sporadical patient being presented for randomization. Early in 1986, this informal working party became an official 'Committee for Prenatal Diagnosis' of the Dutch Society of Obstetrics and Gynaecology. At the same time a comprehensive set of minimum requirements prepared by the 'working party'

which a obstetrician has to fulfil before being allowed to perform antenatal diagnosis by means of amniocentesis *and* chorionic villus biopsy, was adopted and ratified by the Society (see Appendix 11.3) and presented at the third CVS Conference in Strasbourg (Jahoda 1986).

Meanwhile, all centres in the Netherlands agreed on a uniform method of reporting, thus not only ensuring completeness of data for the whole of the country, but also absolute comparability of the results and complications. Data on the number of procedures and follow-up results for the Netherlands in 1986 are shown in Tables 2.2 and 2.3 (not all annual reports 1987 had been received at the time of writing this thesis) (Kloosterman 1988).

Table 2.2 Chorionic villus sampling (CVS) and amniocentesis (AC) in the Netherlands, 1986. Totals, abnormal results and follow-up results as reported by 6 centres (Amsterdam, Arnhem, Groningen, Leiden, Rotterdam and Utrecht).

	CVS	AC	Total
	1288	4018	5306
abnormal result			168 (3.2%)
'spontaneous' abortion < 16 wks	35 (2.7%)[1]		
'spontaneous' abortion ≥ 16 < 28 wks	26 (2.1%)	50 (1.2%)	
preterm delivery < 37 wks	47 (3.6%)	138 (3.4%)	
perinatal death	8 (0.7%)	36 (1.0%)	

Table 2.3 Antenatal-diagnosis-utilization rates in the Netherlands 1980-1986 for the indication advanced maternal age (≥36 years) (Kloosterman 1988).

	number of children from women ≥38 years	number of children from women ≥36 years	number of amniocenteses or CVS	utilization rate (indication maternal age)
1980	2946	–	641	21.7%
1981	3076	–	717	23.3%
1982	3195	–	834	26.1%
1983	3103	–	1059	35.0%
1984	3271	–	1564	47.8%
1985	–	9090	2507	27.6%
1986	–	12090	3584	29.6%

Note: In the Netherlands the maternal age limit for antenatal diagnosis was lowered from 38 to 36 years, officially as per 1 January 1985, but already anticipated upon earlier.

Also before 1985 the expected date of delivery was often taken as the cut-off point for maternal age instead of 18 weeks gestational age being used thereafter. This explains the 'inflated' utilization rates in 1984 and 1985.

Moreover, all centres regularly report to the various world-wide registries (WHO Registry, Jackson's 'Fetal Loss Registry', Mikkelsen's 'Cytogenetics

[1] Percentages over n = 1280 (8 CVS cases were lost to follow-up).

Registry'), showing the overall Dutch antenatal-diagnosis-utilization rate to be relatively high (30% for the indication 'advanced maternal age' in 1986) (Mikkelsen 1986).

2.1.5 Chorionic Villus Sampling in the northern provinces of the Netherlands

After having found out that the aspiration cannulas used by Ward and his team (1983) were commercially available ('Trophocan' from Portex™), we stopped experimenting with prototypes of infant endotracheal tubing and various obturators. The Rotterdam team (Jahoda, Sachs and Vosters) enthousiastically offered us the opportunity of watching several of their villus sampling procedures being performed clinically. By then (early 1984) we had already started practising the procedure on consenting women scheduled for termination of pregnancy, just prior to vacuum aspiration and had been greatly assisted by the hospitality and cooperation provided by clients and doctors of the 'Stimezo' in Groningen (Dr. P. Kersten and his team). The laboratory side of CVS was taken up by the Department of Human Genetics, State University of Groningen: (Prof.Dr. G.J.P.A. Anders; at present Prof.Dr. Ch.C.M. Buys), Dr. M. Breuning being initially in charge, followed by Dr. A.S.P.M. Breed who put the whole project on a firm cytogenetic basis. Initially we felt we should honour requests for diagnostic CVS in pregnancies intended to continue to term after having reached a 75% level of proficiency in terms of successful sampling and subsequent karyotyping, keeping 1 January 1985 in mind as a target date.

However, we were overtaken by a few strong requests for CVS by women who had previously given birth to a chromosomally abnormal child and were very keen on the exclusion of a recurrence as early as possible. Thus we performed our first clinical CVS on 6 November 1984, with slightly less experience behind us than was to be officially recommended later on (see Appendix 11.3).

Not being sure of the volume of response and knowing that at the outset, with the personnel and equipment at our disposal, we could only handle a limited number of cases per week, we first let the obstetricians in the provinces of Groningen, Friesland, Drenthe and Overijssel know in writing that we were ready to conduct clinical CVS at our hospital. This was later followed by a similar letter to general practitioners and midwives in the same area (see Appendix 11.5).

Figures 2.1, 2.2, 2.3 and 2.4 give a general overview of the antenatal diagnostic activities at our clinic throughout the years, starting at the introduction of 2nd trimester amniocentesis in 1974. Note that in the Netherlands a modified set of indications was introduced in 1985, the major changes being: 1. maternal age limit lowered from 38 years to 36 years and 2. widening of the indications for amniotic fluid AFP measurement. Moreover, the automatism of karyotypic analysis in the case of previous history of neural tube defects in 2nd or 3rd degree kinship was given up. (see Appendix 11.2 for a complete overview of the indications for amniocentesis or chorionic villus sampling which have been officially operative in the Netherlands since 1 January 1985).

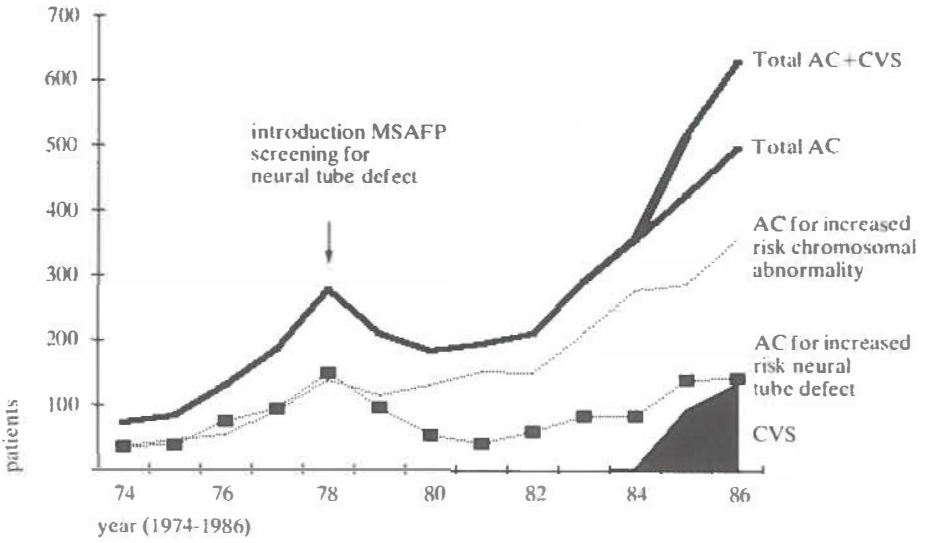


Figure 2.1 Amniocentesis (AC) and chorionic villus sampling (CVS) 1974-1986, Department of Obstetrics and Gynaecology, University Hospital, Groningen, the Netherlands.
(MSAFP = maternal serum alpha-fetoprotein).

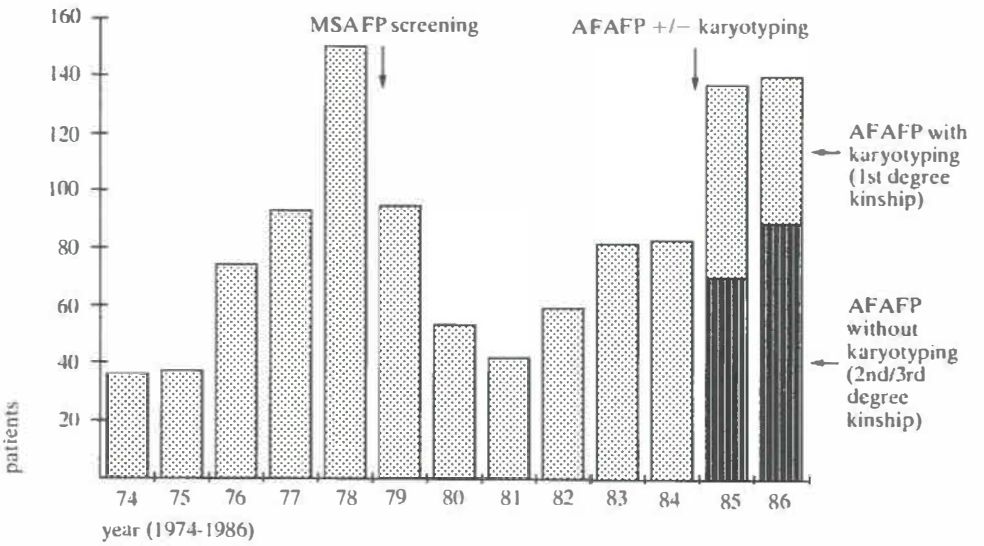


Figure 2.2 Amniocentesis (AC) for increased risk of neural tube defect 1974-1986, Department of Obstetrics and Gynaecology, University Hospital, Groningen, the Netherlands.
(MSAFP = maternal serum alpha-fetoprotein; AFAFP = amniotic fluid alpha-fetoprotein).

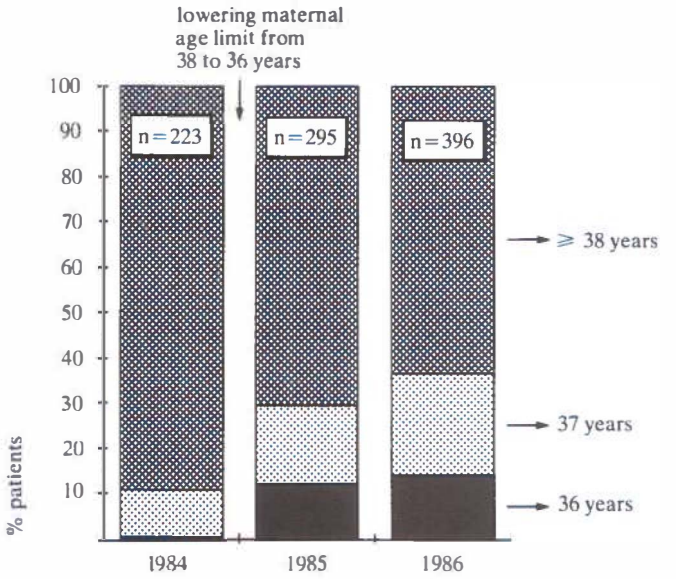


Figure 2.3 Percentage contribution to antenatal diagnosis (chorionic villus sampling and amniocentesis) by maternal age groups 36 years, 37 years and 38 years, 1984-1986, Department of Obstetrics and Gynaecology, University Hospital, Groningen, the Netherlands.

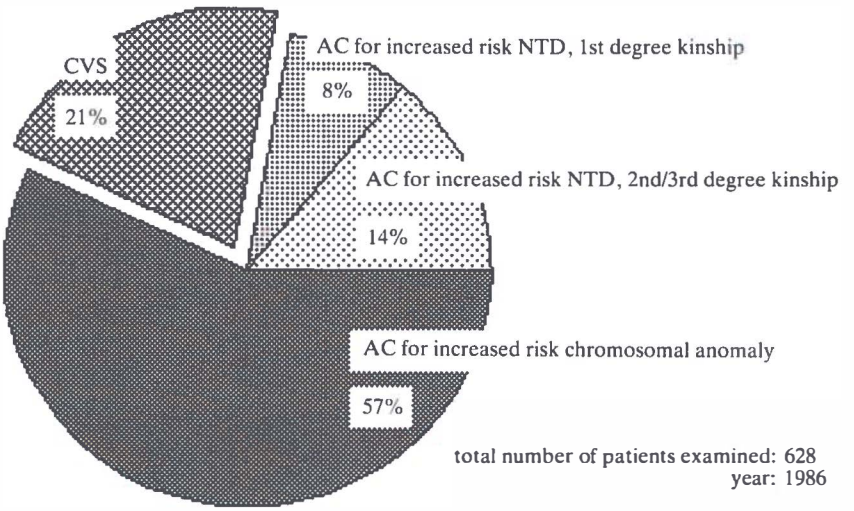


Figure 2.4 Percentage distribution amniocentesis (AC) and chorionic villus sampling (CVS) in 1986, Department of Obstetrics and Gynaecology, University Hospital, Groningen, the Netherlands.
(NTD = neural tube defect).

2.1.6 Future prospects

When are we going to find a 'perfect' test: completely safe for mother and child giving a quick and 100% accurate result and generally applicable in the first few weeks of pregnancy? Not for a long time so it seems.

Research on early pregnancy throphoblastic emboli in peripheral maternal blood has raised the hope of being able to use venous blood for fetal karyotyping one day (Goodfellow et al 1982, Covone et al 1984, 1986). Adinolfi, in his address to the 1987 Chicago CVS Conference, declared that the results of his research in this field did not hold a realistic promise for another breakthrough in antenatal diagnosis, like the Brambati/Simoni one in 1983: fetal cells were too few and too poor in quality to allow reliable chromosomal analysis. Jackson (1987/23) responded however, upbeat:

> *'He (Adinolfi) showed us how the dream of finding little trophies floating in mommy's bloodstream is just a dream so far. Nobody has any hard evidence that they are floating there with any predictable consistency, and if they are, that they are analyzable or that they have been separable from mommy's cells by techniques so far utilized. That is not to say that some midnight Coulter counter operator isn't going to unlock the mystery of the traveling trophoblast tomorrow after dark....'*

This issue might become rather academic, however, if the accuracy of sexing and karyotyping fetal lymphocytes in maternal blood could be improved. At least 'harvesting' them as early as the 7th week of gestation might not be the major problem anymore (Selypes and Lorencz 1988).

Work on pre-nidation genetic analysis is more promising, though its use will be restricted to pregnancies which are genetically at high risk, e.g. fetal sexing for X-linked disorders after in vitro fertilization and before embryo transfer by means of a Y-chromosome specific DNA probe for couples who object to post-implantation termination of pregnancy (McLaren 1985, West et al 1987).

Great improvement, apart from continuing the expansion of our diagnostic arsenal (e.g. metabolic disorders), would be made in the laboratory by means of developing far less labour-intensive methods for investigating amniotic fluid and chorionic villus material. Meanwhile limited laboratory capacity is reason for not lowering the maternal age indication to well below the 36 years limit. Yet this is, to a certain extent, a necessity if we wish antenatal diagnosis to have a sizeable public health impact in terms of detection efficiency of chromosomal disorders (Haveman 1985, Crandell et al 1986), unless, of course, screening procedures are going to be utilized to supplement these diagnostic techniques (e.g. maternal serum alpha-fetoprotein concentration as a marker for trisomy 21, see also section 6.1.).

Overall there is a strong impression that for the time being the scene on the diagnostic side is firmly set. Basic changes do not lie directly ahead but we will see some extensions to the applicability of existing methods, such as CVS moving into the 2nd trimester (Szabó et al 1984, Pijpers et al 1988) and vice-versa amniocentesis into the 1st trimester (Ashmead et al 1987, Cordone et al 1987, Hanson et al 1987), as well as the development of alternative approaches such

as the transvaginal route for a retroverted uterus with a posterior placenta (Jackson 1986/16).

Apart from continuing efforts to try to develop the 'ideal' suction cannula, further activities will be aimed at specific issues, such as Rhesus sensitization by CVS; what to do with positive cervical smears before sampling, etc., all in order to assess, and hopefully reduce the risks inherent to this kind of invasive procedure as much as possible.

In the public health sector, discussions will focus on the cost-benefit aspects of loosening-up the age-limit restrictions (Hagard et al 1976, Huether et al 1983). Debates on psychosocial aspects are heating up, for example on the (un)desirability of having this kind of 'imperative' technologies at our disposal and their impact on individuals (Tijmstra 1987); or on CVS' potential for misuse, e.g. abortion in the case of the undesired sex (Campbell 1984). Misuse or not, it has not only happened in China (Anshan 1975), but it is 'established practice' now in other countries as well (Singh 1985). In Denmark the parents are not informed of the sex of the fetus until after the 14th week of pregnancy (Hahnemann 1986).

Medico-legal aspects of antenatal diagnosis are receiving also increasing attention e.g. issues of 'wrongful birth', 'wrongful life', and 'wrongful parenthood' to be settled in the USA (Fletcher 1985). *'Just wait till it reaches Europe'* (Milunsky 1985). In Germany, a case of disputed parenthood was even solved by CVS (Gross-Wilde 1986).

Prevention by elimination (termination of pregnancy) is one thing, but prevention by treatment is by far the most preferable way. Work on pre-nidation gene therapy is not only fascinating, but it also holds some approach to practical applicability e.g. gene correction for Lesch-Nyhan's disease (Smithies 1987).

The more down-to-earth obstetrical, CVS-related issues which should be addressed in the future, according to Blakemore (1986), are:

- fetal loss
- intrauterine infection
- threatened abortion
- amniotic fluid leakage
- amniotic band syndrome
- sensitization to Rhesus and other antigens
- fetal malformations and other teratogenic effects

and, because part of the placenta is removed during sampling, theoretically:

- intrauterine growth retardation
- premature labour
- premature rupture of membranes
- abruptio placentae
- placenta accreta/increta
- placenta praevia
- pregnancy-induced hypertension.

Final remarks

Chorionic villus sampling has been embraced swiftly and enthousiastically by patients and doctors alike and a word of caution has only been heard occasionally (Wyatt 1983, 1985; Hecht et al 1984, Hecht and Kaiser-McGaw Hecht 1985). Nevertheless CVS has not yet stood the test of time. In terms of numbers by the end of 1987, with over 35,000 CVS cases since the introduction of CVS into clinical practice (Jackson 1987/23), one may assume that it has lived up to expectations. But although the overall picture seems to be reassuringly characterized by a lower CVS related abortion risk than initially thought, we are also faced with questionable reliability, which is being increasingly substantiated by reports about false-positive results and, very unsettling, recently even by some false-negative results (discussed in section 5.1).

For many couples whether or not to choose CVS in favour of amniocentesis is often a kind of 'seatpants decision', where objective pros and cons outweigh each other. But the occurrence of false positive CVS results, without knowing for sure which ones to overlook and pass as normal and which ones to distrust, actually necessitates confirmation of all abnormal results by amniocentesis and is very disturbing. This might not only diminish the attractiveness of CVS over amniocentesis, but might also demote CVS from the ranks of a diagnostic procedure to those of a screening method (i.e. requiring further tests to verify its results).

Moreover, the first reports of false-negative results are genuinely alarming and might ultimately prove to be lethal for the test. If further substantiated, we inevitably have to conclude that the placenta falls too far short of the amniotic fluid in reliabity for reflecting the genetic make-up of the fetus. In the years ahead we will probably hear more underground rumblings about CVS; parts of this thesis will already familiarize you with the sound. Amniocentesis has not aged, CVS has not yet come of age.

2.2 Aim of the study

'You can't kick nature in the ribs and get away with it', so what does the new test of first trimester chorionic villus sampling (CVS) add to the existing methods of antenatal diagnosis, especially second trimester amniocentesis? People in the northern provinces of the Netherlands are not easily swept away by novelties so we assumed that a substantial number of women would still prefer amniocentesis to CVS.

Therefore, the first aim of the study was to assess the merits and disadvantages of CVS, in order to provide answers to questions regarding general aspects of the test, i.e.

- safety and complications;
- accuracy;
- applicability and acceptability.

We shall try to answer these questions not by randomized controlled trial, but by strictly following-up a 2-year cohort of the first 427 women who applied for antenatal diagnosis early enough in pregnancy to have both options (CVS or amniocentesis) open.

The second aim of the study was to obtain data from research into some specific aspects of CVS, i.e.

- feto-maternal haemorrhage caused by the test;
- fetal motility before and after CVS;
- placental pathology and fetal outcome;
- psychosocial aspects.

This was accomplished by specifically investigating these aspects in 4 subgroups of various size (188, 10, 50 and 20 women, respectively), not only merely to complete the overall picture, but also to see which other, not so obvious problems might lie ahead (and hoping, of course, that this setting would prove to be serendipitous as well).

3 PATIENTS

3.1 Patients: general description, referral patterns and response rates

Our preliminary activities were described in section 2.1.5 and resulted in a total of 427 applications for chorionic villus sampling (CVS) during the 2 year investigation period 4 November 1984 (date of the first CVS) till 28 October 1986 (date of the last CVS described in this study). These women were referred either specifically for CVS or for 'antenatal diagnosis' in general, but in the latter case early enough for a possible CVS (at 10 weeks + 6 days gestational age at the latest). Time permitting, they were sent information on CVS and amniocentesis (AC) at their home address (Appendix 11.4) and were booked for intake at our hospital which consisted of ultrasound examination, followed by an interview and, if indicated, a gynaecological examination.

At intake we stressed 4 points of information on CVS:

1. The procedure-related abortion risk was not (yet) exactly known but was 'probably higher' than compared with 2nd trimester amniocentesis (this AC added risk was quoted as being less than 1%). In the course of 1985 we changed 'probably higher' into 'slightly higher'.
2. As CVS does not test for neural tube defects, it should be supplemented maternal serum alpha-fetoprotein screening in the 16th week if required.
3. There was a small chance of sampling or analysis failure (to which we later added the occasional need for confirmatory amniocentesis in the case of ambiguous laboratory results).
4. There was no exact knowledge (as yet) about any possible long-term effects, such as e.g. fetal growth retardation or premature rupture of the membranes (we later added that there had been no evidence for this either).

This ultimately resulted in 216 (50.6%) women who underwent CVS (section 5.1) and in 211 (49.4%) who did not for various reasons (section 5.2).

Let us look into the distribution of the total group of 427 women: where did they come from and who referred them? More specifically, what was the response rate in the respective provinces of the northern Netherlands and did referral patterns change with the introduction of CVS, as compared with the period prior to 1984, when only amniocentesis was available. Table 3.1 gives a breakdown of our CVS study group from November 1984 to October 1986 (n = 427), according to province of residence and referrer. The data are summarized graphically in Figure 3.1. As midwives may also refer through general practitioners, the latter's figures are slightly inflated.

Table 3.1 Referral pattern of study group for chorionic villus sampling (CVS) (n = 427).

GP = General Practioner
M = Midwife
S = Self
O = Obstetrician
Dpt = Department of Obst. & Gynae., Univ. Hosp., Groningen
G = Geneticist

Province	GP	M	S	O	Dpt	G	Other	Total	%
Groningen	44	6	13	33	15	3	1	125	29.3%
Friesland	56	0	1	46	7	4	0	114	26.7%
Drenthe	50	3	3	56	4	6	0	122	28.6%
Overijssel	16	4	1	36	1	1	1	60	14.1%
Elsewhere	4	0	0	2	0	0	0	6	1.4%
Total	180	13	18	173	27	14	2	427	

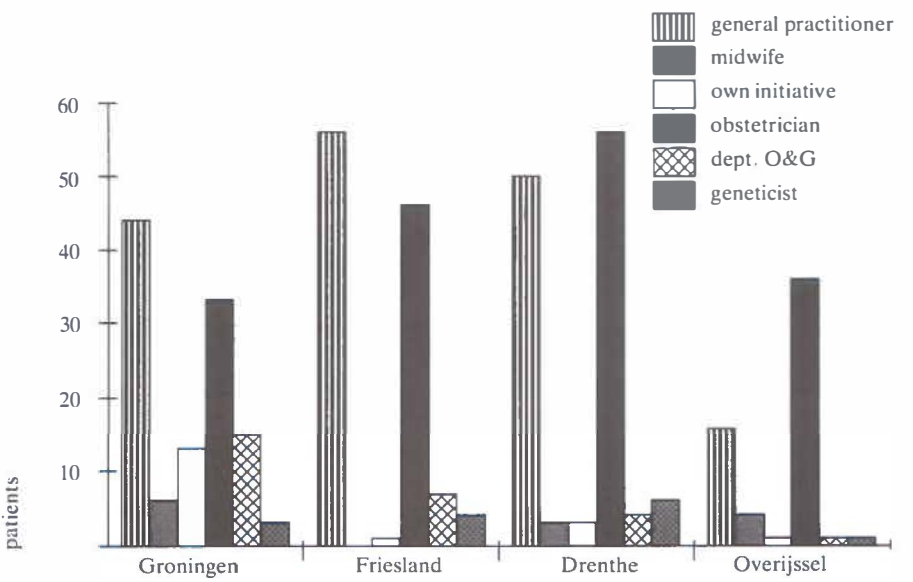


Figure 3.1 Referral pattern of study group chorionic villus sampling (n = 427), according to the province of residence and referrer, Nov. 1984 - Oct. 1986, Department of Obstetrics and Gynaecology, University Hospital, Groningen, the Netherlands.
Note: midwives may also refer via general practitioners; category 'other' is omitted.

1st Health care echelon (general practitioners, midwives and 'self' i.e. on the patient's own initiative) and 2nd echelon (regional and university specialists, geneticists and 'somebody else') contributed practically fifty-fifty to this group, with 211 and 216 referrals, respectively (49.4% and 50.6%).

To illustrate the trend of contribution per province over the past years, Table 3.2 shows the number of women per province expressed as a percentage of the total number of women referred for antenatal diagnosis. Province contribution data from 1979 to 1984 were obtained from the annual reports of the 'Stichting voor Erfelijkheidsvoorlichting' (St.Erf.) '79-'81, '82, '83 and '84, respectively (there were no data available for 1985 and 1986 at the time of writing this thesis; our CVS study group will have to act as a substitute). The 1987 figures have been incorporated.

Table 3.2 Province contribution to amniocentesis (AC) and chorionic villus sampling (CVS) according to patient's province of residence, all indications.

	1979 n=97 AC	1980 n=197 AC	1981 n=199 AC	1982 n=220 AC	1983 n=311 AC	1984 n=351 AC	'84-'86 n=427 CVS study group	1987 n=702 AC + CVS
Province	%	%	%	%	%	%	%	%
Groningen	25	38	34	32	34	31	29	28
Friesland	32	27	32	30	27	29	27	25
Drenthe	19	20	13	17	17	22	29	21
Overijssel	21	11	16	17	16	17	14	20
Elsewhere	4	5	5	5	6	2	1	4

Turning to a more specific discussion on referral patterns and actual response rates, we will address two questions:

I. Did referral patterns change in the northern provinces of the Netherlands with the introduction of chorionic villus sampling?

II. What is the real response rate per province amongst women 'eligible' for antenatal diagnosis and did that change as well?

Ad I: *Referral patterns for antenatal diagnosis in the northern provinces of the Netherlands*

The referral figures for amniocentesis according to the referrer are only available for '81, '82 and '83 (annual reports St.Erf.).

A break-down of the figures over these years together with our CVS study group is shown in Table 3.3, grouped in first and second health care echelon referrers. The data are summarized in Figure 3.2.

Table 3.3 1st and 2nd Echelon referrals for amniocentesis (AC) and chorionic villus sampling (CVS), all indications.

1981	1982 AC n = 200	1983 AC n = 220	'84-'86 AC n = 313	CVS study group n = 427
1st echelon	64 (32%)	68 (31%)	99 (32%)	211 (49%)
2nd echelon	136 (68%)	152 (69%)	214 (68%)	216 (51%)

We can indeed see that the referral ratio between these groups, 1st and 2nd echelon, shifted globally from a 1 : 2 to a 1 : 1 situation.

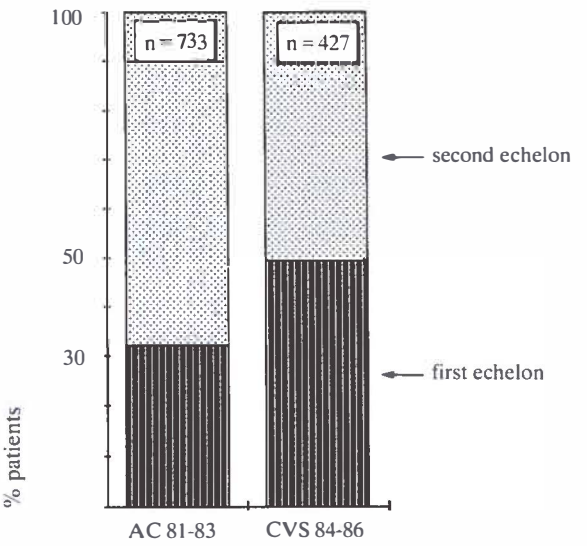


Figure 3.2 Percentage of women referred for amniocentesis (AC) 1981-1983 (n = 733) and chorionic villus sampling (CVS) Nov.1984-Oct.1986 (n = 427) by 1st echelon (general practitioner, midwife or own initiative) and 2nd echelon (obstetrician, geneticist or our own department), Department of Obstetrics and Gynaecology, University Hospital, Groningen, the Netherlands.

One might raise the objection that if the amniocentesis figures for '84-'86 had been available, they might have offset the CVS shift, possibly resulting in an overall pattern that would have remained at roughly the same 1-2 ratio. Therefore we have added our 1987 annual report figures[1]:

[1] Jaarverslag Antenatale Diagnostiek 1987, Kliniek voor Obstetrie en Gynaecology, Academisch Ziekenhuis, Groningen.

1987	CVS	AC	Total
1st echelon	109	142	251
2nd echelon	108	300	408

Once again there is a 50-50 ratio for CVS and a 32-68 ratio for amniocentesis, resulting in an overall 38-62 ratio.

In conclusion, the strong CVS referral pattern by general practitioner has caused the overall antenatal diagnosis referral ratio between 1st echelon and 2nd echelon referrers to shift from 30-70 to 40-60, and has meant that the responsibility for initial genetic counselling has shifted increasingly towards these 1st instead of 2nd echelon workers. Apart from the introduction of CVS as an important contributing factor to shifted referrals patterns, there might be another one: the '81-'83 group is not completely comparable with the '84-'86 CVS group and the 1987 group, because the maternal age indication dropped from 38 to 36 years in the meantime, i.e. older women are more likely to be under specialist's attention. Also, are women who have been referred by specialists, preselected towards amniocentesis in some particular way? (see section 5.2.2).

Ad II: (*Actual*) *response rate per province for age-indication*.

Table 3.2 shows that the contribution of each province to the total antenatal diagnosis figure remained fairly constant over the years. To approximate the actual response rate among women 'eligible' for antenatal diagnosis in the respective provinces, we need to know the number of women who were 'viably pregnant' in a particular period of time. As the precise figures are not known, the response rates in a particular year are usually given against the number of live births in the 'index group' during that same year. The following year would be a more logical choice considering the 40 week gestation period in humans. Here we will use a maternal age of $\geqslant$36 years as an index. Before breaking down the number of pregnant women in these provinces during the period under investigation, let us first turn to the Netherlands as a whole, where there was a total of 184,513 live births in 1986, with a steady rise (8.4%) from 170,246 in 1983 (source Netherlands Central Bureau of Statistics, CBS, The Hague). The contribution made by women of 35 years and older (women were grouped 35-39 years, 40-44 years, etc.) rose from 13,254 children (= 7.8% of total) in 1983, to 16,777 (9.1% of total) in 1986 (26.6% rise!).

A breakdown by province and by age for 1985 and 1986 was kindly provided by the CBS. These data are shown in Table 3.4, slightly readjusted to suit our purpose.

Table 3.4 Live births according to province and age of the mother on 31 Dec. '85 and 31 Dec. '86, respectively (source CBS).

maternal age in years	Groningen		Friesland		Drenthe		Overijssel[1]	
	1985	1986	1985	1986	1985	1986	1985	1986
36	113	154	136	155	72	91	220	273
37	90	104	74	95	74	66	170	161
38	55	65	87	65	43	48	129	121
39	48	40	53	57	31	43	110	100
40	27	41	30	35	18	22	52	66
41	23	11	22	19	14	6	31	37
42	10	3	12	20	2	6	29	29
43	3	8	8	11	2	3	14	18
44	4	2	3	7	2	2	10	5
45	1	1	2	4	2	3	6	4
46	0	4	1	0	2	1	3	1
47	0	0	1	0	0	0	2	1
48	0	0	0	0	1	0	1	0
49	0	0	2	1	0	0	0	0
50	0	1	0	0	0	0	1	0
≥51	0	0	1	2	0	0	0	3
Total	374	434	432	471	263	291	778	819
Age adustment minus 1/3 of the 36y group*	38	51	45	52	24	30	73	91
	336	383	387	419	239	261	705	728

* A number of the women who delivered or became pregnant in the first few months of a particular year will have had their birthday late in the same year and will therefore have to be excluded on the grounds of being too young to meet the age criterion for antenatal diagnosis (which states that a woman must be at least 36 years of age at 18 weeks of gestation). For uniformity, we agreed nationally to subtract 1/3 from the 36-year-old category. The mathematical formula suggests an unrealistic exactness as e.g. deliveries are not usually evenly spread over the year. Calculation by pregnancy calender shows that 1/3 is a suitable approximation.

In our CVS study group, we had 315 couples with a maternal age of ≥36 years and 1 couple with both maternal and paternal age indication (42y + 60y), totalling 316 (1 couple with a paternal age of ≥55 years was omitted).

A breakdown of this age-indication group according to province is given in Table 3.5.

[1] Noord-Oost Polder included.

Table 3.5 Contribution to chorionic villus sampling (CVS) per province for maternal age ⩾36 years

Province	Total number and %-CVS	Women eligible for antenatal diagnosis '85+'86 from Table 3.4	% contribution of all eligible women age indication
Groningen	95 (31%)	719	13%
Friesland	78 (25%)	806	10%
Drenthe	96 (30%)	500	19%
Overijssel	40 (13%)	1433	3%
Elsewhere	3 (1%)	–	–
not clear	4 (2%)	–	–
Total	316		

Unfortunately the table does not tell us the response rates for antenatal diagnosis in general, i.e. CVS *and* amniocentesis. To keep things relatively simple and to be able to make a comparison with the national Dutch figures, we will analyze the 1986 data only. As we saw in Table 3.4, the number of children born in 1986 to mothers of ⩾36 years was 383 for Groningen, 419 for Friesland, 261 for Drenthe and 728 for Overijssel + the Noord-Oost Polder.

The number of women with the indication 'maternal age ⩾36 years' who underwent amniocentesis was 101 in Groningen, 78 in Friesland and 54 in Drenthe (source Stichting Erfelijkheidsvoorlichting, Groningen[1]. For CVS the figures were 30, 24 and 32, respectively. By adding the total number of women eligible for antenatal diagnosis as shown in Table 3.4 and dividing the results by the latter figures, the maternal-age response rates for these three provinces in 1986 were found to be: Groningen 131:383 = 34.2%, Friesland 102:419 = 24.3% and Drenthe 86:261 = 32.9%[2], together good for a response rate for the northern provinces of the Netherlands as a whole of 319 : 1063 = 30.0%. Figure 3.3 shows these in comparison with overall 1986 national response rate of 29.6% (data per province were not available) (Kloosterman 1988).

[1] Overijssel is left out as a substantial number of women is referred elsewhere.

[2] This is a slight correction on the provincial response rates given in our annual report 1987.

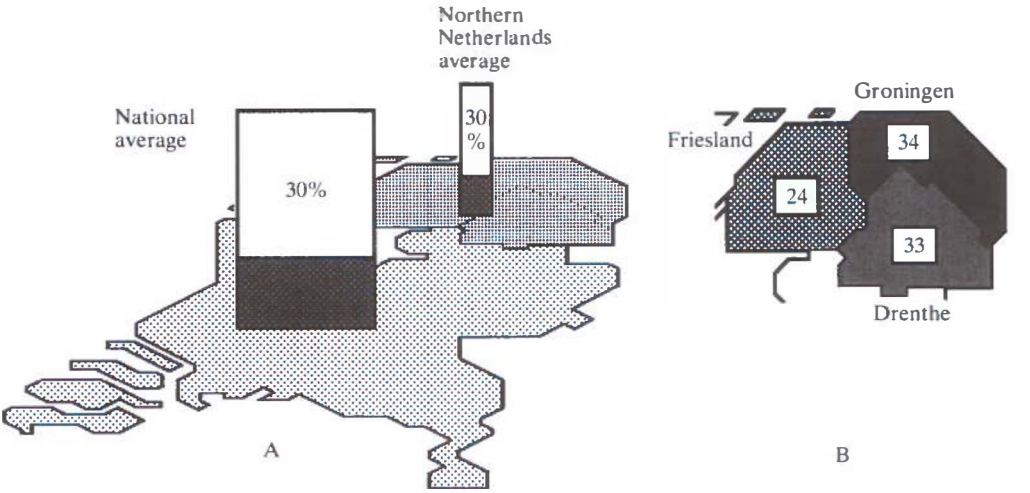


Figure 3.3 Response rates of women ⩾36 years for antenatal diagnosis (amniocentesis and chorionic villus sampling) in 1986 for indication 'advanced maternal age' in the north of the Netherlands, proportionally compared with the Dutch national average (A) and the provinces Groningen, Friesland and Drenthe (B), respectively.
Note: Overijssel is omitted as patients are also referred to other medical centres.

Discussion

The 1978 data presented in a study by Thomassen-Brepols (1985) show antenatal diagnostic response rates in the maternal age group ⩾38 years to be 23%, 19% and 23% for Groningen, Friesland and Drenthe, respectively, against a national average of 20%. The latest overall national average she reported (not broken down per province) was 31% for 1984.

We see a. that the rates in Groningen and Drenthe are consistently above the national average, whereas the rates in Friesland are not only well below, but also lower in relation to 8 years ago; b. that CVS did not cause an increase in overall utilization of antenatal diagnosis and c. that CVS is also unlikely to have attracted a substantial number of women who would otherwise have declined antenatal diagnosis altogether.

Alhough the Netherlands may have a relatively high antenatal diagnosis utilization rate world-wide (Mikkelsen 1986), we are still way off the 50% response rate which is thought to be necessary (in combination with lowering the age limit to 35 years) if we wish to reduce the number of Down syndrome infants born by the year 2000 by 10% (Haveman 1985). Crandell et al (1986) has calculated that even if the maternal age-limit is lowered to 30 years in

combination with a utilization rate of 50%, it would still only mean that 27% of Down syndrome pregnancies would be detected. Therefore, alhough we have lowered the age limit and might expect to catch up a bit via the 36 and 37 year group after a slow start in 1985, the contribution cannot be expected to amount to much (before 1985 the age limit was 38 years; see section 3.3 for age distribution in the study group). Of course, a relatively low response rate per se is not lamentable, provided that the non-responders who declined antenatal diagnosis had been properly informed. Thomassen-Brepols (1983, 1985) has suggested that a substantial proportion of this non-response group was the result of poor counselling at that time and that the existing response rates can easily be improved upon: optimal counselling could even result in a 75% response rate. Similarly but to a far lesser extent, the same reason probably lies behind what happened in the early eighties, considering the rather substantial increase in response rates in the northern provinces of the Netherlands as compared with the 1978 data mentioned above. On the other hand, 75% still seems to be an illusion and we doubt whether a lack of information really plays a major role nowadays. In any case, the 1985 ruling of the Dutch Medical Disciplinary Board makes it rather unattractive for medical workers not to give adequate genetic counselling. (Anonymus 1986a, 1986b; Meeuwissen 1986).

3.2 Diagnostic cases (n = 216) and drop-outs (n = 211)

Of the 427 patients who were referred to our centre during the two-year study period (November 1984 - October 1986) at an early enough stage to be able to choose between chorionic villus sampling (CVS), amniocentesis or neither, 216 underwent CVS and 211 did not.

The main groups of reasons for not wanting, or undergoing CVS are listed in Table 3.6. Figure 3.4 gives a schematic overview. Details on this drop-out group are presented in section 5.2.

Table 3.6 Reasons in drop-out group (n = 211) for not undergoing chorionic villus sampling (CVS).
(NTD = neural tube defect, IUD = intrauterine device).

1. Woman expressed personal preference for amniocentesis		69 = 33%
– smaller abortion risk	59	
– test includes NTD	10	
2. Had a spontaneous abortion		56 = 27%
– before intake	13	
– after intake	43	
3. Was advised amniocentesis by us		48 = 23%
– too large-for-date	9	
– recent bleeding	8	
– technical problems at CVS	7	
– multiple gestation	6	
– IUD related	4	
– history neural tube defect	2	
– vaginal infection	2	
– chemo/radiotherapy	2	
– assorted problems	8	
4. Woman changed her mind altogether		36 = 17%
– declined all antenatal diagnosis	27	
– preferred termination of pregnancy	9	
5. Unclassified		2

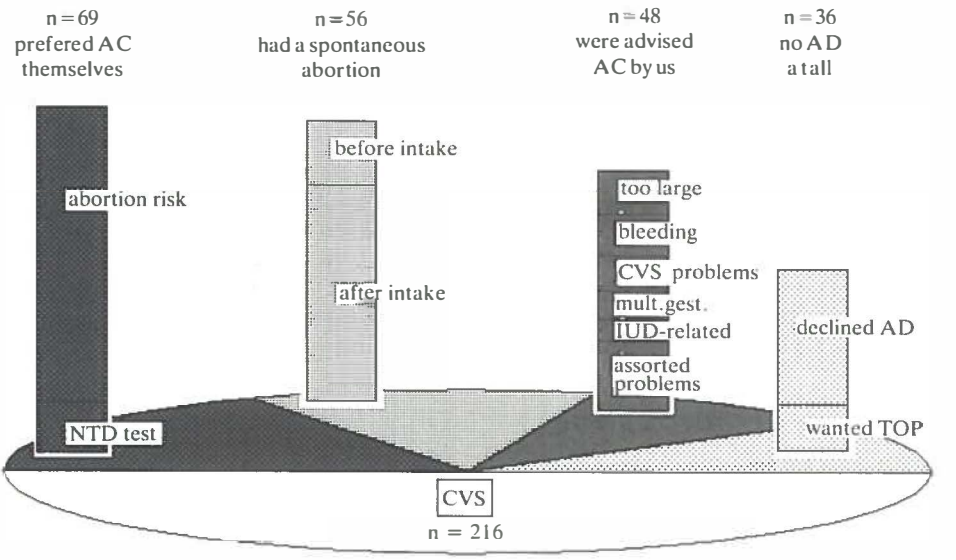


Figure 3.4 Distribution of reasons for not undergoing chorionic villus sampling (CVS), Nov. 1984 - Oct. 1986 (n = 211), Department of Obstetrics and Gynaecology, University Hospital, Groningen, the Netherlands.
(AC = amniocentesis, AD = antenatal diagnosis, IUD = intrauterine device, Mult.gest. = multiple gestation, NTD = neural tube defect, TOP = termination of pregnancy).

Discussion

Roughly speaking, half of the women indeed underwent CVS; the other half who did not were split up into a number of rather equal parts depending on the reason: 'preferred amniocentesis', 'had an abortion' or 'miscellaneous'. This 50-50 ratio was present straight from the beginning and did not change appreciably throughout the study period (Mantingh et al 1986b). It is possible that the abortion risk group who preferred amniocentesis might become smaller as soon as the exact risk is known (Perry et al 1985, Keppels et al 1986), but the other group will remain fairly large because the contributing factors, such as e.g. intercurrent abortion, desire to test for neural tube defects, or preferring to terminate the pregnancy for emotional reasons after all, are not expected to change. The time and effort per patient spent on CVS during intake and sampling already compares very unfavourably with amniocentesis, moreover, part of it will turn out to have been 'wasted' anyway. (Mantingh et al 1986a). On the other hand, quite a number of women who get caught up in the medical circuit e.g. and undergo one or more ultrasound examinations to verify their non-viable pregancies, followed by vacuum-aspiration, would otherwise possibly have had a complete abortion at home without any medical intervention whatsoever.

Further ups and downs of this drop-out group are discussed in section 5.2.

3.3 Indications and Contra-indications

The indications for antenatal diagnosis in our study group of 427 women who applied for chorionic villus sampling (CVS) are listed in Table 3.7. If a combination of indications was present, the patient was classified under the condition with the highest recurrence risk. In the case of e.g. maternal age and a previous child with trisomy 21, however, the patient was classified under prior chromosomal anomaly (although the maternal age risk could easily supersede the previous history risk), as she would have come for antenatal diagnosis anyway, no matter how old she was (The cystic fibrosis (CF) patient was the only exception: besides a previous child with CF, she also had one with trisomy 18).

Table 3.7 Indications for chorionic villus sampling (CVS) (n = 427) (CF patient counted twice)
(NTD = neural tube defect; MSUD = maple syrup urine disease)

			n	%
Age			317	74.1%
Maternal age	≤36y	2[1]		
	36	61		
	37	65		
	38	80		
	39	45		
	40	31		
	41	16		
	42	8		
	43	3		
	44	3		
	45	1		
	≥46	0		
Paternal age ≥55 years		1		
Both maternal and paternal age (42 and 60 years)		1		
Parental chromosomal rearrangement			10	2.3%
Former child with chromosomal anomaly			61	14.3%
Carrier X-linked disease			11	2.6%
– Duchenne's muscular dystrophy (also for DNA)		4		
– Becker's muscular dystrophy (no DNA: fam. not informat.		1		
– haemophilia A		2		
– sex-linked hydrocephaly (same patient)		2		
– fragile X		1		
Former child with metabolic disease			7	1.6%
– Hunter		1		
– Zellweger (same patient)		2		
– Sanfilippo		1		
– Pompe		1		
– MSUD		1		
– CF (also for DNA)		1		

[1] Both women 35 years + 8 months at the time of CVS.

(For DNA analysis[1]	5	1.2%)	
(Duchenne's muscular dystrophy	4)		
(cystic fibrosis	1)		
Parental anxiety		18[2]	4.2%
– Down's syndrome in family	8		
– cong. abnorm. previous child	4		
– sudden infant death syndrome	1		
– bad forecast astrologer	1		
– working with handicapped children	1		
– Down's syndrome with friends	1		
– no specific reason, other than 34y+9m and 35y+0m	2		
Miscellaneous		4	0.9%
– chemotherapy	2		
– NTD child (+ mat.age)	1		
– NTD 2nd degree (our mistake: mat.age 20y)	1		
(radiotherapy + mat.age 39y, counted as age)			
Total		428	

Contra-indications at the time of intake for CVS, apart from the 'evident' ones (such as threatened abortion, twins, wrong indication etc.), were only sporadically present. They are listed below (and discussed in more detail in section 5.2).

Absolute contraindications		4
– cervical stenosis	1	
– cervicitis	2	
– fibroids + heparine treatment	1	
Relative contraindications		8
– diabetes mellitus	1	
– IUD in situ or just removed	4	
– time needed for family investigation	1	
– after exconisation + 2nd infert.	1	
– adipositas 4+	1	

Discussion

We did not consider unfavourable conditions such as e.g. uterine fibroids or a strongly retroverted uterus with a placenta situated posteriorly, as contraindications per se. Echoscopic (intra)uterine features and dimensions sometimes seem to change completely overnight.

In general, Scrimgeour's list of (contra)indications is a detailed, yet practical overview of all conditions one could possibly encounter when facing the choice between CVS or amniocentesis or both (Scrimgeour 1985):

[1] Not counted twice, hence brackets.

[2] Of these 18 'parental anxiety reasons' 11 indeed underwent CVS or AC; the other 7 did not. Result: 10 normal results, and one Turner's syndrome!

Contra-indications for CVS

Absolute:
1. Diagnosis not possible by CVS
2. Offer refused
3. Dead fetus
4. Gestation over 10 weeks duration (menstrual age)
5. Placenta not located echoscopically
6. Retroverted uterus with a fundal or posteriorly located placenta
7. Stenosed cervix

Relative:
1. Expertise/experience not available
   a. obstetric
   b. laboratory
   c. personnel
   d. financial
2. Rhesus immunization
3. Multiple pregnancies
4. Vaginal infection
5. Multiple fibroids
6. Currently bleeding
7. IUD in situ
8. Previous infertility
9. Previous recurrent abortions
10. Religion
11. TOP doubtful after positive result
12. Termination of pregnancy not legal
13. Fear of risk of procedure

Indications for amniocentesis following CVS:

1. Unsuccessful CVS
   a. obstetric
   b. laboratory

2. Confirmatory in the case of
   - mosaicism
   - metabolic disorders
   - unbalanced rearrangements (especially deletions)
   - raised maternal serum alphafetoprotein at 16 wks
   - double check on result

Most of these are straightforward enough. Some could be overcome by the abdominal CVS approach instead of the transcervical (e.g. cervical stenosis), while others are debatable (e.g. 10 weeks gestational age limit). An important issue concerns the need for a cervical swab for culture and sensitivity at intake and subsequently cancelling the CVS on account of the time factor if it is found to be positive).

4 METHODS

4.1 Ultrasound

At intake and during sampling we used an ALOKA™ SSD - 256, equipped with a 3.5 MHz linear array transducer. This was not because we considered this ultrasound set-up to be the most suitable for chorionic villus sampling (CVS), it just happened to be available. Moreover, experience in the build-up phase of the service was gained using a linear array machine, so we stuck to it when starting CVS on a clinical basis, although initial reports often suggested a real-time sector scan to be superior for this kind of work (i.a. Jahoda et al 1984).

During 1986 an ACUSON™ sector scanner was introduced at our department so we switched ultrasound intake procedures to this machine. We kept relying on the ALOKA during CVS though. We checked routinely at intake: – position of the uterus – localisation of the placenta – crown-rump length of the fetus – placental insertion of the umbilical cord – presence and position of the yolk sac and for any particulars such as multiple gestations, fibroids, haematoma etc., see illustrations Figures 4.1 – 4.4 (see Chapter 10 for full details on the ultrasound checklist at intake).

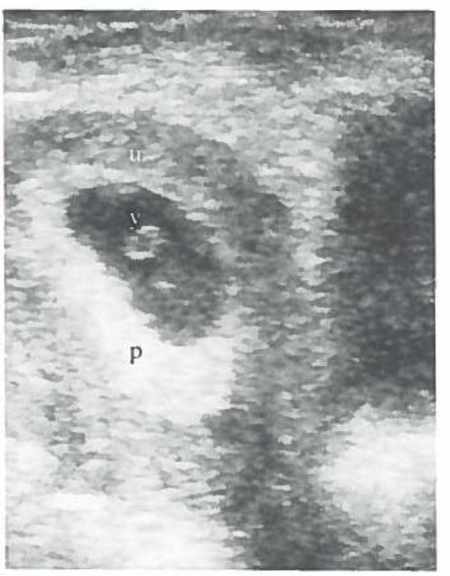


Figure 4.1 Longitudinal ultrasound scan at intake (7 weeks + 4 days gestation) showing anteverted uterus (u), posterior placenta (p) and yolk sac (y).

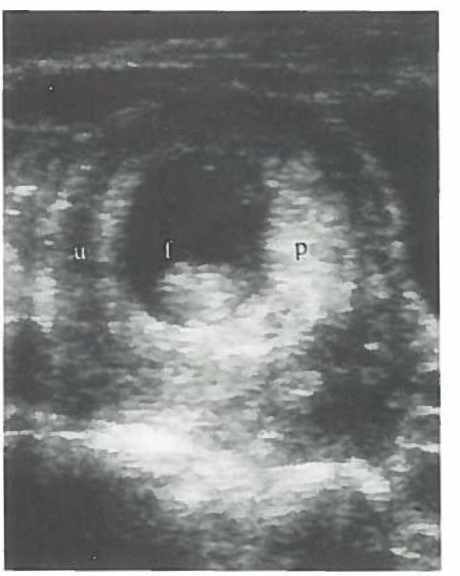


Figure 4.2 Longitudinal ultrasound scan at intake (9 weeks and 2 days gestation) showing anteverted uterus (u), anterior placenta (p) and fetal parts (f).

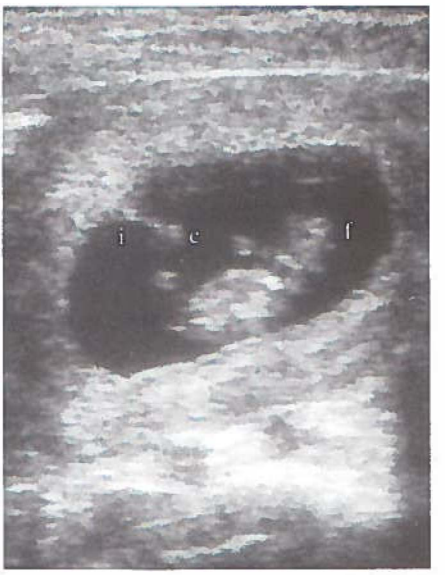


Figure 4.3 Transverse ultrasound scan at intake (10 weeks + 0 days gestation) showing fetus (f) and a right/fundal insertion (i) of the umbilical cord (c).

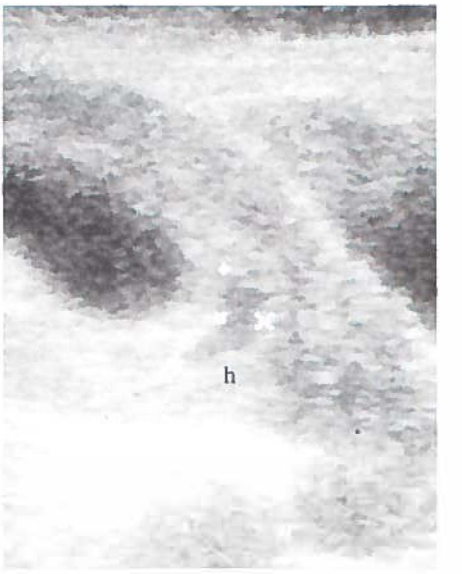


Figure 4.4 Longitudinal ultrasound scan at intake (7 weeks + 2 days gestation) showing an echolucent area (14x16mm) at the internal os, probably haematoma (h).

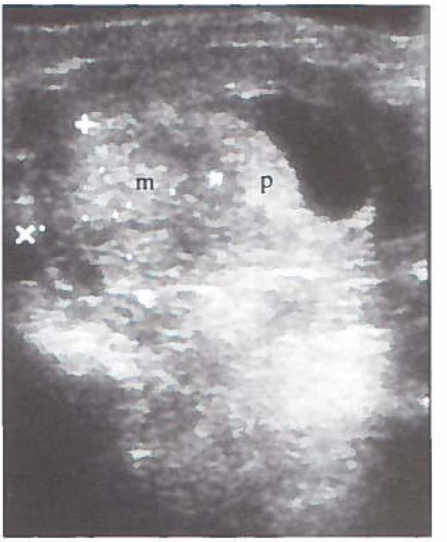


Figure 4.5 Longitudinal ultrasound scan at intake (8 weeks + 6 days gestation) showing a 50x40 mm posterior wall myoma (m), just behind the placenta (p).

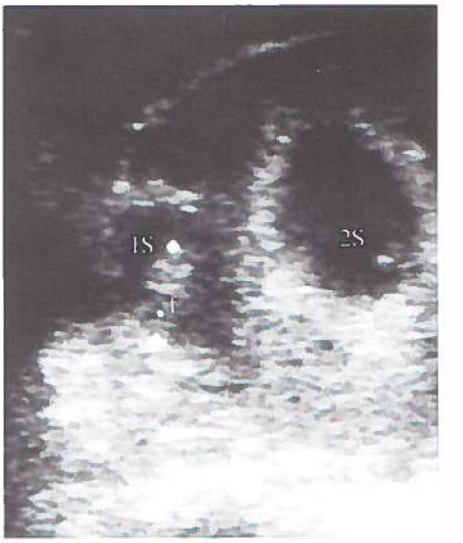


Figure 4.6 Oblique ultrasound scan at intake (8 weeks + 5 days gestation) showing first sac (1s) (normal fetus lying outside plane of scanning) and second sac (2s) containing non-vital fetal parts (f).

4.2 Sampling

Chorionic villi were obtained around the 10th gestational week. We used the poly-ethylene Portex™ cannula ('Trophocan'), 18 cm long and 1.5 mm external diameter with an aluminium obturator malleable according to the expected intrauterine route. This cannula was introduced transcervically under ultrasonic control and strict antiseptic conditions. After having manoeuvred the catheter into the proper placental area to be biopsied, the obturator was removed, after which a 30 ml syringe filled with approximately 2 ml RPMI[1] was attached to it. Suction (10-15 ml) was applied on withdrawal of the cannula and chorionic villi flushed into a Petri dish. The sample was then examined microscopically to check whether it was suitable for analysis.

This procedure was identical to the method described by i.a. Simoni et al (1983), Ward et al (1984) and Jahoda et al (1984). We only occasionally used the rigid silver-metal King's College cannula when we saw on our echoscope that the plastic Portex catheter was slipping away from the intended place of biopsy after removal of the stylet. We did not favour this cannula (and other metal ones of the same type) as our first choice, as we considered its rigidity could increase the likelihood of decidual damage and subsequent bleeding on withdrawal.

Figures 4.7, 4.8 and 4.9 illustrate these various steps.

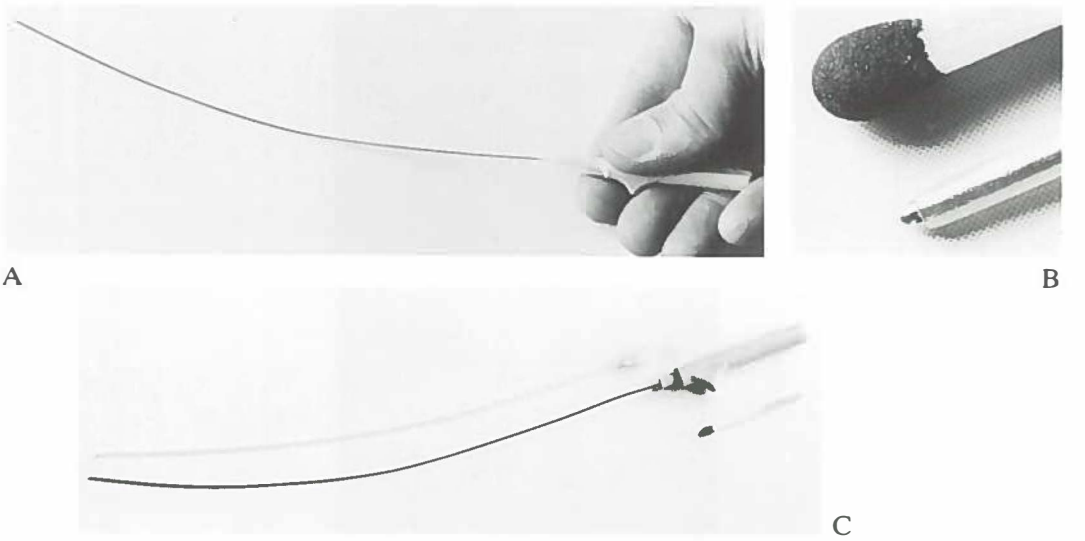
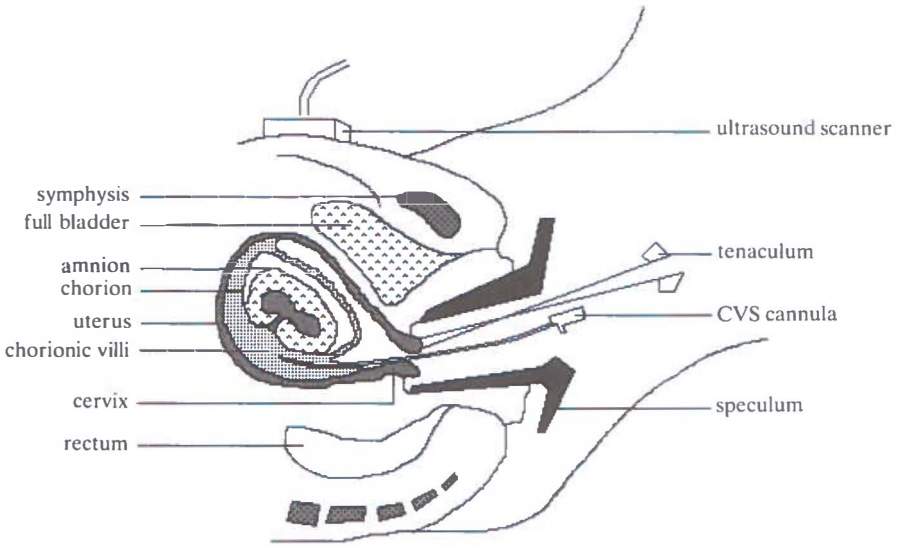
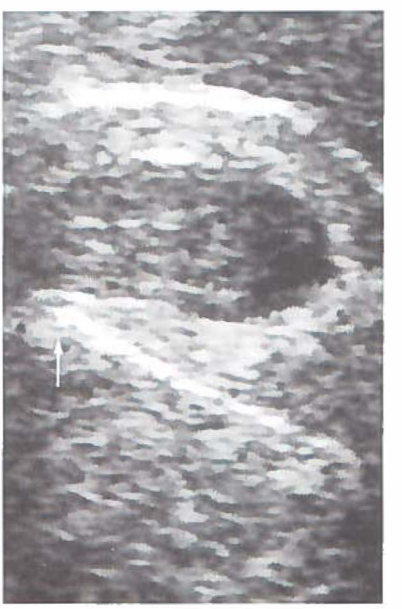


Figure 4.7 The Portex™ system for chorionic villus sampling ('Trophocan') consisting of a malleable aluminium obturator and a poly-ethylene cannula (18 cm long, 1.5 mm external diameter): A. obturator in situ, B. close-up of cannula-tip, C. obturator removed.

[1] RPMI 1640-medium / 1% heparin (1000U/ml)



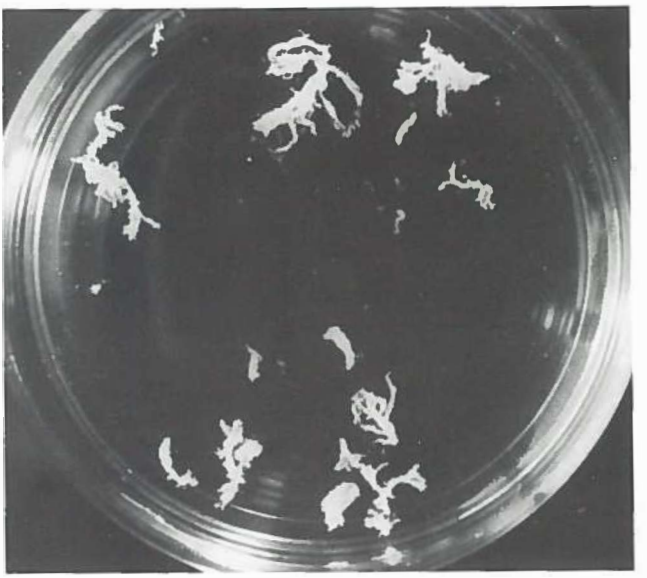
A



B

Figure 4.8 Transcervical chorionic villus sampling using the Portex ™ aspiration cannula ('Trophocan'): A. diagrammatic representation and B. corresponding ultrasound image showing cannula tip at site of sampling (arrow).

A



B

Figure 4.9 Chorionic villi flushed out after sampling, floating in RPMI-filled Petri dish (A) and at low power magnification (B). (Department of Human Genetics, University Groningen).

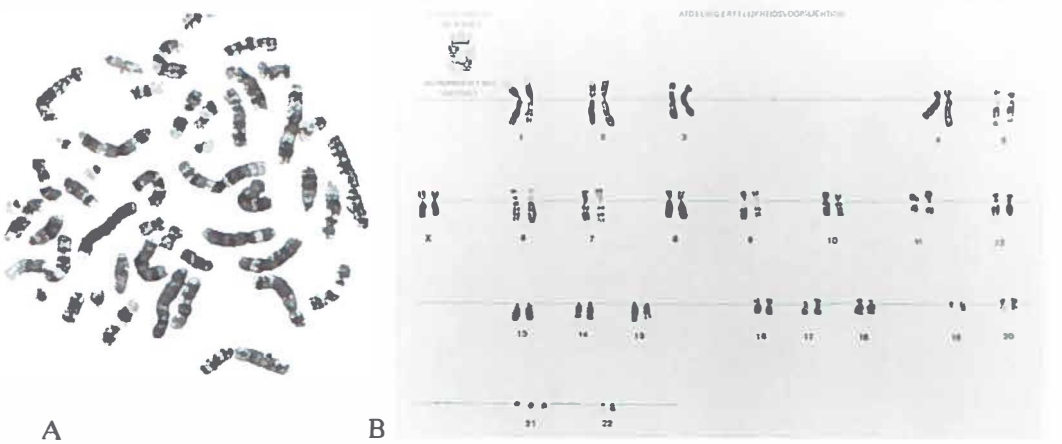
4.3 Karyotyping

Chorionic villi aspirated in the syringe containing RPMI were subsequently transferred to two Petri dishes: one containing 2.3 ml RPMI 1640 for direct chromosome preparation and one containing 1.8 ml RPMI plus 0.5 ml fetal calf serum for 24 hour incubation. Chromosomal analysis was then performed according to the Simoni technique (Simoni et al 1984). Usually chromosomal abnormalities were based on the analysis of at least 14 G-banded metaphases (see Figure 4.10).

All karyotypes were analyzed at the cytogenetic laboratory (Dr. A.S.P.M. Breed) of the Department of Human Genetics (Prof. Dr. G.J.P.A. Anders; at present Prof. Dr. Ch.C.M. Buys), University of Groningen, except for a few that were performed in conjunction with enzymatic analysis for metabolic disorders at the Department of Clinical Genetics, Erasmus University, Rotterdam (Dr. E.S. Sachs and Dr. W.J. Kleyer).

DNA analyses for Duchenne's and Becker's muscular dystrophy were carried out at the Department of Human Genetics (Prof.Dr. P.L. Pearson), University of Leiden.

The one cystic fibrosis DNA analysis was conducted at the Department of Human Genetics, University of Groningen (Prof.Dr. Ch.C.M. Buys).



A B

Figure 4.10 Chorionic metaphase as seen at high power magnification (A) and example of laid-out chromosomes showing trisomy 21 karyotype (B) (Down's syndrome, arrow points at the extra chromosome no. 21). (Department of Human Genetics, University Groningen)

5 RESULTS

5.1 Results of 216 diagnostic cases

During the 2-year period under discussion (November 1984 - October 1986), we performed chorionic villus sampling (CVS) on 207 women and referred 9 women to the Diaconessen Ziekenhuis in Arnhem (Dr. M.D. Kloosterman and Dr. G.C.M.L. Christiaens) in August 1986 due to a staff shortage.

The results of the total of 216 CVS are given in Table 5.1. Practically all karyotypings were performed at the Cytogenetic Laboratory (Dr. A.S.P.M. Breed), Department of Human Genetics (Prof.Dr. G.J.P.A. Anders; at present Prof.Dr. Ch.C.M. Buys), State University, Groningen (see section 4.3 for exceptions).

Table 5.1 Cytogenetic result (n = 216)

Normal karyotypes	
46,XX, normal female	93
46,XY, normal male	91
inversions	1
balanced translocations (like parent)	4
Total	189 = 94%[1]
abnormal karyotypes (chronologically; inversions and balanced translocations excluded)	10 = 5.0%
69,XXX	
69,XXY	
47,XY,+del(16)	
94,XXYY,+21,+21	
47,XY,+21	
46,XY(70%)/92,XXYY(30%)	
46,XY/46,XY,6p+(12/2 metaphases)	
47,XY,+21	
47,XY,+21	
47,XY,+21(90%)/48,XY,+21,+9(10%)	
Sampling failed or no karyotype obtained (Groningen 14, Arnhem 1)	15 = 6.9%[2]

[1] Based on 202 karyotypes (15 failed + 3 done on amniotic fluid indication 'metabolic' + 10 abnormalities = 28; 189 + 28 = 217 (1 twin)).
[2] Based on 216.

5.1.1 True-positive results

Chromosomal abnormalities in chorionic villus material were either confirmed by subsequent 16th week amniotic fluid cell karyotyping or by fetal tissue analysis after termination of pregnancy.

True abnormal karyotypes with follow-up results are listed in Table 5.2 and numbered in chronological order.

Table 5.2 True abnormal cytogenetic results (n = 216)
(TOP = termination of pregnancy)

caseno.	karyotype	followed by	fetal tissue
1	69,XXX	TOP	69,XXY
2	69,XXX	TOP	69,XXX
5	47,XY,+21	TOP	47,XY,+21
8	47,XY,+21	TOP	no follow-up[1]
9	47,XY,+21	TOP	47,XY,+21

5.1.2 False-positive results

Abnormal karyotypes with discrepant follow-up results are listed in Table 5.3.

Table 5.3 False-positive and discordant cytogenetic results.
(TOP = termination of pregnancy; AC = amniocentesis)

caseno.	karyotype	followed by	fetal tissue
3	47,XY,+del(16)	TOP	46,XY
4	49,XXYY,+21,+21	TOP	47,XY,+21
6	46,XY(70%)/ 92,XXYY(30%)	AC	46,XY
7	46,XY/46,XY,6p+	AC	46,XY
10	47,XY,+21/ 48,XY,+21,+9(10%)	TOP	47,XY,+21

We had no false-negative results.

Discussion (sections 5.1.1 and 5.1.2)

Strictly speaking we had 5 correct diagnoses of chromosomal abnormalities (case no. 8 is considered to be correct because a false-positive trisomy 21 is indeed not impossible, but highly unlikely, see later), as well as 5 discrepancies in the 2-year period under investigation. None of these had any serious consequences, as the chromosomal diagnosis was either rejected by the

[1] Was admitted elsewhere for vaginal bleeding after CVS and had a vacuumcurettage as soon as the karyotype was known. Tissue karyotyping of the products of conception sent to us, failed.

amniocentesis result (nos 6 + 7) or turned out to be abnormal anyway after termination of pregnancy (nos 4 and 10), with one exception: case no. 3, extensively described in 'Prenatal Diagnosis' (Breed et al 1986). We had already strongly suspected a discrepancy here, based on the bizarre 'non-viable' karyotype in combination with a perfectly normal fetus at ultrasound examination. The couple rejected a confirmatory 16th week amniocentesis, however, because they wanted 'to have the issue solved in the first trimester of pregnancy'. Obviously the chromosomal make-up of chorionic villus material is not necessarily identical to the fetal karyotype and potentially this may have grave implications. Indeed, discrepancies between chromosomal constitution of chorionic villi and fetal tissue have been reported several times and among them rather unnerving ones. In a December 1986 update (Breed et al 1984), using Groningen and Arnhem data combined, we arrived at a total of 6 confirmed and 2 yet to be confirmed discrepancies (including 1 straightforward trisomy 18!), out of 16 chromosomal aberrations diagnosed on chorionic villi. These, and other data from major reports elsewhere, are summarized in Table 5.4.

Table 5.4 Comparison of chromosomal unbalanced diagnoses from chorionic villus sampling between 5 major centres

	no. of diagnostic cases	no. of aberrations	no. of discrepancies	predictive value[1]	95% confidence limits
Jackson 1985	460	20 (4.4%)	2	90%	70- 97%
Mikkelsen 1985	1401	83 (5,9%)	9	89%	81- 94%
Sachs 1985	350	11 (3.1%)	–	100%	74-100%
Simoni 1986	1000	47 (4,7%)	8	83%	70- 91%
our data 1986	350	16 (4.6%)	6	62%	39- 82%
Overall	3561	177 (4.7%)	25	86%	80- 90%

The last two mosaicisms awaiting further confirmation mentioned above were found to be false-positive too, bringing the number of false-positive results to 8 and lowering the 62% predictive value even more.

Another major follow-up of chorionic villus sampling (CVS) results from three other centres in the Netherlands was recently published by Leschot et al (1987). They arrived at 15 discrepancies from a total number of 481 chorionic villus karyotypings (3.1%), most of them mosaicisms, but also a trisomy 11.

Discrepancies do occur. It is not a matter of 'not looking properly', or 'not daring to dismiss them and pass them as normal karyotypes' as this phenomenon is sometimes shrugged off. They are for real and should be acted on by way of second trimester confirmatory amniocentesis, unless the couple concerned

[1] Predictive value = number of true positive : total number of positives.

wishes otherwise. On the other hand, if all abnormal karyotypes should have to be confirmed this way, what is, strictly speaking, the use of CVS as a diagnostic test anyway? Obviously doubtful and would not we therefore be wise to regard it as a screening procedure only (i.e. requiring further tests to verify its results)? Nevertheless it has appeared that some chromosomal anomalies are more likely to represent a true picture of the fetus than others. Trying to put this likelihood into exact figures for the respective types of chromosomal abnormalities, in other words precisely stating the predictability of individual chromosomal aberrations, is beyond the scope of this thesis. But generally speaking, 'deletions' should be highly suspected and will probably turn out to be normal when seen in combination with an echoscopically normal fetus. The same applies to mosaicisms with varying outcomes of the confirming amniocentesis depending on the type of mosaicism. Finally, for numerical abnormalities it is highly improbable that they will turn out to be normal, but again not impossible as we have seen.

What about the possibility of false-negative results? Initially, we held a rather firmly opinion that false-negative resulys would not occur. At the Chicago 1987 CVS Symposium an overview was presented, setting the tone for a rather disheartening discussion on the reliability of CVS in general. Table 5.5 summarizes a compilation of the experience at major centres presented at that meeting (Milano, Genua, Chicago, Philadelphia) and other false-negative case reports from the litterature (a.o. Eichenbaum et al 1986, Martin et al 1986).

Table 5.5 Compilation of false-negative chorionic villus sampling results reported up until September 1987
(DP = direct preparation of chorionic villus material TC = tissue culture of chorionic villus material AC = amniocentesis CC = cordocentesis)

No.	DP	TC	AC	CC	Fetus	Recheck villi in retrospect
1.	46,XY(20)	–	–	–	47,XY,+21(17) (blood)	46,XY(88)/ 47,XY,+21(1)
2.	46,XY(15)	–	–	–	blood:46,XY, ((del(18) (q21-qter))(100) fibroblasts: -id-(50)	46,XY(34)
3.	46,XY	XO/XXY	XO/XXY	–	XO/XXY	–
3.	46,XY	XO/XXY	XO/XXY	–	XO/XXY	–
5.	46,X.	XY/XXY	–	–	XY/XXY	–
6.	46,X.	XO/XY	–	–	XO/XY	–
7.	46,X.	+18	–	–	+18	–
8.	46,XX	47,XX,+21 (71%)	repeat CVS: 46,XX AC:46,XX	47,XX,+21 +21(1/70)	47,XX, in+21 3 tissues	

In a recent NIH study, 2 cases of trisomy 21 could be traced as having been overlooked at CVS, as well as 3 errors in fetal sexing (Jackson 1987).

This does not look too good. The only consistent feature seems to be that in the patients in whom chorionic villi were analyzed in combination with subsequent tissue culture, instead of after direct preparation only, chromosomal abnormalities were detected that were not present in the directly prepared karyograms. Doing tissue cultures in conjunction with the direct preparations of chorionic villus material in all CVS cases, however, might just substitute the solution by another problem, because earlier experience with CVS also showed that the likelihood of false-positive results, such as mosaicisms, increases the longer one cultures, not to mention the demands it would put on laboratories in terms of manpower and facilities.

Another observation, also presented at the above meeting (Jackson 1987), concerned six spontaneous abortions after CVS with normal CVS results but abnormal fetal tissue typing: +3, +9, XXY+2, +16, −+16, +15, respectively! Probably the CVS situation on that occasion was best characterized by someone despairing: 'It gives you the creeps, can't you trust anything?'.

Our first paper regarding reliability of CVS as a diagnostic test (Breed et al 1985) was initially titled : 'CVS not to be trusted?', but we dropped it as being a bit too speculative at that time, considering the small number of cases we had. The more experience we will gain however, the more likely it seems that this claim can be substantiated by convincingly documented discrepancies. At least for the time being we stand by our conclusions as presented at the Strasbourg 1986 CVS conference (Breed et al 1986).

1. The reliability of the CVS procedure should be evaluated on the basis of the predictive value of the test. The acceptability of the procedure should, among other aspects, be discussed against this background.
2. All chromosomal abnormalities diagnosed in CVS specimens should be checked in post-abortal tissue or by amniocentesis.
3. Prior to CVS, patients should be informed about the possibility of diagnostic discrepancies.

Finally, the necessity to have any abnormal CVS karyotype confirmed by amniocentesis not only diminishes the attractiveness of CVS over amniocentesis, but might also move CVS from the ranks of diagnostic procedures and label it as a screening method, putting it on a par with e.g. maternal serum alpha-fetoprotein screening i.e requiring further tests to verify its results (Mantingh et al 1986a). Some authors even go as far as considering CVS results to be some 20 times more unreliable than amniocentesis (Callen et al 1987). Of course, as we have already said in the introduction, certain advantages as experienced from the individual point of view of the couples concerned, might well outweigh this (see section 6.4).

5.1.3 Failures

As mentioned in section 5.1, we failed to obtain a chromosomal diagnosis in 15 women (6.9%), 14 at our department and one in Arnhem, giving a success rate of 93.1%.

These 14 failures consisted of 11 sampling failures (i.e. no villi, or unsuitable villi obtained and qualified as such during sampling by the cytogeneticist or his laboratory technician) and 3 laboratory failures (i.e. specimen initially passed as suitable, but at karyotyping found to contain no, or non-analyzable metaphases).

5.1.3.1 Sampling failures

No women were a priori excluded from chorionic villus sampling (CVS) on the basis of particular ultrasound findings at intake (such as fibroids or an extremely retroverted uterus with the placenta located on the posterior wall), provided that embryonic development was in accordance with the gestational age and excluding multiple gestations (with the exception of one couple with a twin pregnancy who insisted).

We considered sampling to have taken place as soon as the cannula had echoscopically clearly passed the level of the internal os, regardless of whether aspiration had taken place or not, in other words when 'a pass had been made' into the uterine cavity and not when actual aspiration had taken place. An overview of these unsuccessful samplings, with particulars and the probable reason for failure, is given in Table 5.6.

Table 5.6 Particulars of unsuccessful chorionic villus samplings and reasons (n = 11)
(US = ultrasound; FTD = full term delivery; Ind.Ab. = Induced abortion; AC = amniocentesis; X0 = Turner's syndrome; Sp.Ab. = spontaneous abortion)

Case no.	Details of sampling	Outcome
20	Confusing US (cord anterior, placenta posterior)	FTD
27	Difficult insertion cannula, no evident reason	Ind.Ab. after AC (X0)
45	Cannula perpendicular on placenta	FTD
167	Placenta out of reach, fibroid onstructing	Sp.Ab.
243	Poor view	FTD
251	Poor view	FTD
273	No clear reason	FTD
293	Poor view, probably liquor aspiration	FTD
303	Tight cervical canal, fibroid obstructing	FTD
369	Placenta out of reach, fibroid obstructing	FTD

(no particulars available in one case)

An additional 7 women underwent a successful 2nd CVS session after the 1st had failed; reasons for the initial failure being less clear-cut than in the group above.

5.1.3.2 Laboratory failures

These cases will only be mentioned briefly here as this subject is not within the scope of this thesis. As said above, the main reason was that no (analyzable) metaphases were present because the poor quality of villi had been insufficiently realised during sampling. For the sake of completeness, the data are summarized with a few particulars in Table 5.7.

Table 5.7 Unsuccessful karyotyping chorionic villus samplings (n = 3)
(AC = amniocentesis)

Case no.	Particulars
79	1st Session 43 mg villi acceptable villi, but only 2 ?metaphases XX. 2nd and 3rd Session poor villi (3 and 28 mg resp.), AC: 46,XY.
131	1st Session 23 mg 'reasonable' villi, but 1 metaphase XX only. 2nd Session 20 mg good villi: XX.
402	1st Session 37 mg 'reasonable' villi, but no metaphase 2nd Session 30 mg good villi: 46,XY.

Discussion of failures

Although several samplers proudly announce their 'nearly', 'almost' or 'practically' 100% success rates, failure or success will mainly be determined by the kind of criteria applied for accepting or rejecting candidates for CVS, provided a physician enters the field with sound basic experience (see Appendix 10.3).

In retrospect, we could have more than halved our failures by not having started the procedure in case nos 20, 167, 243, 251, 293, 303 and 369 (see section 5.1.3.1). Probably the most important factor predicting failure or success while sampling is a particular kind of 'feel' Jackson described in relation with the increased likelood of intra-uterine trauma (and subsequent fetal loss): '*There is usually a noticeable difference in the "feel" of the procedure to the sampling obstetrician. This is sometimes reported as suggesting that the catheter is not following normal tissue planes or passages and that some poorly explained difficulty is occurring relative to the guidance of the catheter to an appropriate sampling site*' (Jackson 1986/17). After CVS, of course, the suitability of the sampled villi for analysis criteria must be evaluated meticulously according to Simoni's criteria (Simoni et al 1984).

5.1.4 Short-term follow-up (<28 weeks of pregnancy).

Pregnancy problems after chorionic villus sampling (CVS) were categorized into '(frank) bleeding', 'spotting', 'excessive brownish discharge'[1], 'fever/

[1] Brownish discharge, though separately itemized, was only brought up once as a worrisome single problem, probably because we explain it as a 'normal' occurrence after CVS: portio bleeding from cervical forceps, mixed with iodine.

infection', 'abdominal discomfort' 'amniotic fluid leak' and 'miscellaneous'. The time and duration of any complaint was recorded as accurately as possible in order to distinguish between a. Problems during CVS or immediately following sampling, b. First week problems regardless whether evident at CVS or not, but at least present for more than a few hours to a couple of days and c. Late problems usually, of course, also starting early, but continuing for more than one week, often causing considerable concern to patient and doctor alike.

This information was mainly gathered a. at the time of sampling itself, b. at the time of phoning the results to the patients (on which occasion the problems mentioned above were specifically asked for) and finally c. from the obstetrical - annex delivery records, a copy of which is routinely sent to us by the patient's attending family doctor, midwife or gynaecologist. Moreover, our patients agreed to keep us informed of any intercurrent trouble attended to elsewhere. This, combined with our 100% follow-up after delivery, made us feel confident that nothing deviating from an absolutely normal pregnancy escaped our attention.

The distinctive problems are dealt with separately in sections 5.1.4.1 to 5.1.4.7, in order of frequency of occurrence instead of ordered by potential seriousness.

5.1.4.1 Bleeding after Chorionic Villus Sampling (CVS)

After CVS 21 women had frank vaginal bleeding, 13 complained of spotting only and one patient mentioned excessive brown discharge, giving a total of 35 women out of 216 who experienced some sort of vaginal bleeding after CVS (16.2%).

In 10 of the 21 women with frank bleeding, uterine blood loss was evident directly after sampling. In the other 11 it happened in the course of the first week, usually stopping within a couple of days and only continuing on and off for more than a week in 4 women (for 2, 3, 4 and 4 weeks, respectively). Only three mentioned accompanying abdominal discomfort as well. As regards the 'spotting' and 'discharge' group, in all 14 it occurred in the first week and disappeared quickly, except in 1 patient where spotting persisted for approximately one month, neccesitating admission (also in view of her poor obstetric history). One woman complained of possible leakage of liquor as well, but ultrasound follow-up was normal and in one we had accidentally left a small swab in the vagina.

A few of the 21 women with frank bleeding returned to our department for echoscopical reassurance. Their pregnancies were found to be progressing normally. See scans Figure 5.1, 5.2 and 5.3 a+b, illustrating the considerable amount of blood that can obviously be trapped in the uterine cavity for quite some time before being absorbed or expelled without hindering fetal development. On four occasions we had a worrying quantity of blood in our syringe at aspiration, which invariably turned out to be of maternal origin: HbA.

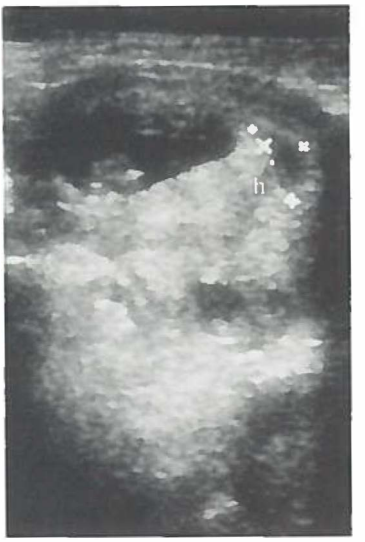


Figure 5.1 Small haematoma (h) directly after CVS at 10 weeks gestation. Slight antepartum haemorrhage, in 31st week and impaired pregnancy outcome in 39th week (2250G, 2.3 centile).
Note: Figure 4.5: same patient.

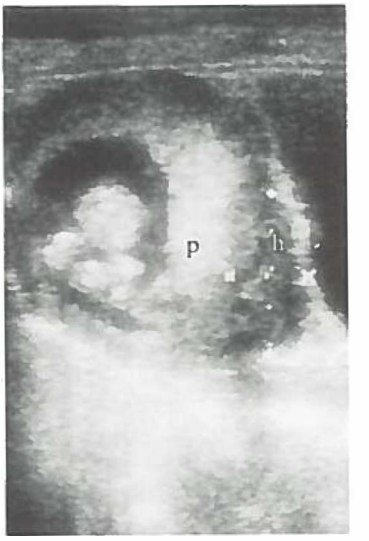
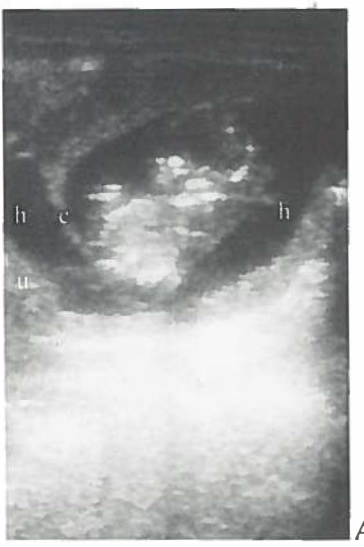
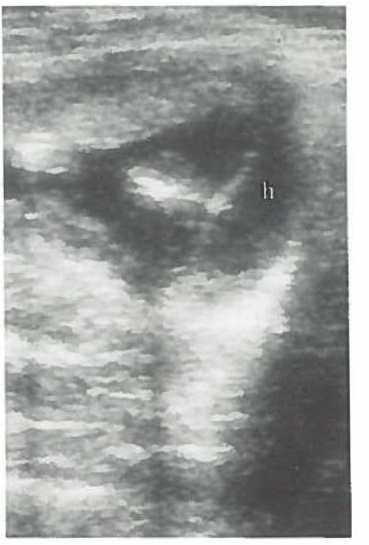


Figure 5.2 Rather echogenic 43x21 mm haematoma (h) behind placenta (p), 4 weeks after CVS at 9 weeks gestation. Vaginal blood loss week 11-13, thereafter uneventful. Outcome normal (4100G).



A



B

Figure 5.3 Big, echolucent haematoma (h) between chorionic plate (c) and uterine wall (u), 4 weeks (A) and 7 weeks (B) after CVS at 9 weeks gestation. Vaginal blood loss since sampling for 4 weeks and heavy brownish discharge for another 4 weeks. Thereafter uneventful. Outcome normal (3520G).

Only 2 spontaneous abortions occurred in this group of 35 women (5.7%), apart from 4 post-CVS women with bleeding who had a induced abortion for abnormal karyotype (1x XXY and 3x trisomy 21). All others delivered full-term infants except for one appropriate-for-date preterm baby of 2430G at 35+0 weeks after rupture of the membranes. P.M. Vaginal blood loss that started 1 week or more after CVS and resulted in a spontaneous abortion is excluded here and discussed in section 5.1.4.2.

Discussion

What consequences does vaginal bleeding have after CVS? There seems to be an easy answer, as from our figures one would say none, at least, no serious ones such as e.g. an increased risk of spontaneous abortion. Nevertheless, bleeding causes concern, sometimes leading to admission to hospital and repeated ultrasound follow-ups. Or, as one patient, who had undergone amniocentesis in a previous pregnancy, complained: '*I experienced more difficulties with CVS than with amniocentesis. Not only was it much more painful, but also because of the uncertainty caused by the bleeding which kept occurring afterwards*'. Reason enough to try to reduce it as much as possible.

Approaching the issue of potentially facilitating and perhaps avoiding factors which result in vaginal bleeding after CVS, we shall try to answer 3 questions regarding how this bleeding relates to various conditions:

A. Does the bleeding relate to previous (obstetric) history, in other words, is there a previous history which is in any way predictive, e.g. previous spontaneous abortions, vaginal bleeding before CVS, uterine abnormalities detected at ultrasound, etc.
B. Does the bleeding relate to the procedure itself, such as e.g. the number of cannula insertions, quantity of villi aspirated, position of uterus, placental localisation, etc.
C. Did the bleeding effect the course of pregnancy unfavourably. Although it might not have influenced the pregnancy unduly in terms of fetal loss, it might be associated with problems such as antepartum haemorrhage, fetal growth retardation, or premature rupture of the membranes.

A summary of these 'obvious' features is listed in Table 5.8, categorized in under the above groups A, B and C.

Table 5.8 'Bleeding' and 'no bleeding' after CVS in association with previous obstetric history, particulars at sampling and pregnancy outcome

		After CVS	
		'Vaginal Bleeding' group (n = 35)	'No Complaints' group (n = 181)
A	A1. Number of women with previous abortions,	9 = 25.7%	40 = 22.1%
	and number of previous abortion per woman	2.0	1.3
	A2. Number of women with vaginal bleeding before CVS	5 = 14.3%	17 = 9.4%
B	B1. Number of women with abnormal ultra-sound at intake[1],	7 = 20.0%	30 = 16.6%
	remaining and relevant abnormality	4 = 11.5%[2]	14 = 7.7%[3]
	B2. Number of women with retroverted uterus	2 = 5.7%	7 = 3.9%
	and also placenta on posterior wall or in fundo	1 = 2.9%	3 = 1.7%
	B3. Number of cannula insertions per sampling	1.7	1.7
	B4. Average weight of villi obtained at sampling	41 mg	38 mg

[1] The 'abnormality' had either disappeared or was 'corrected' at the time of sampling (mainly 'too small or large for dates' and 'abnormalities unreliable due to poor visualization at intake'), thus 3 and 16 women were excluded, respectively.

[2] 2x shadow/haematoma, 1x fibroids and 1x 2nd sac.

[3] 4x shadow/haematoma, 3x fibroid with shadow/haematoma, 2x fibroid, 2x multiple pregnancy, 2x 2nd sac, 1x fibroid with 2nd sac.

		After CVS	
		'Vaginal Bleeding' group (n = 35)	'No Complaints' group (n = 181)
C.	C1. Raised 16th week maternal serum alfa-fetoprotein (>95 centile)	0	2 = 1.1%
	C2. Antepartum haemorrhage	3 = 8.6%	2 = 1.1%
	C3. Premature rupture of membranes (only)	1 = 2.6%	4 = 2.2%
	C4. Premature contractions (only)	0	7 = 3.9%
	C5. Preterm delivery	1 = 2.6%	2 = 1.1%
	C6. Fetal growth retardation	0	4 = 2.2%

Although these differences are not significant and most figures are too small to calculate the added risk of a particular pre-CVS condition, factors such as previous abortion or vaginal bleeding before CVS are apparently unlikely to contribute much to bleeding after CVS. Subsequently, bleeding after CVS is unlikely to contribute much to spontaneous abortion after CVS, as discussed in the next session. There was, of course, some preselection because most of the worst and 'recent' cases of bleeding (i.e. within one week before scheduled CVS) were considered to be threatened abortions and were consequently advised to wait it out and, if all went well, to undergo amniocentesis (see also section 5.2.1).

This applies to group B1 too: (intra)uterine abnormalities were usually not the worst ones.

The 'retroverted uterus' category B2 puts us in a kind of no win situation if the placenta is also situated posteriorly: if the bladder is filled more fully for better echoscopic visualization of the uterine contents, the utero-cervical junction will lie at more of an angle and it will be more difficult for the catheter to negotiate such a bend. This category is therefore underrepresented here.

Group B3 and B4 might, of course, still contain a few women with wide deviations in terms of milligrams of villi sampled, or in terms of the total number of aspirations performed, which have been averaged out here by the large number of women studied. This is not the case, however,: the maximum number of cannula insertions was 3 per session (occurred a total of 20 times) and the number of sessions was not more than 2 (occurred 11 times in total, except for one persevering patient who had 3 sessions), while the maximum

total weight of villi sampled never exceeded 100 mg.

From category C it appeared that the risk for antepartum haemorrhage might be increased when CVS is followed by vaginal bleeding. It is hard to draw any sensible conclusion now about the other factors (but see sections 5.1.5.1 and 5.2.1 which deal with pregnancy problems and abortion as drop-out factors, respectively).

Generally, it seems that post-CVS vaginal bleeding is not comparable with spontaneously occurring bloodloss as a predictory factor for abortion or an otherwise compromised outcome of pregnancy (Editorial 1980).

5.1.4.2 Spontaneous Abortion after Chorionic Villus Sampling

Abortion due to chorionic villus sampling (CVS) has always been the main worry about the test. The exact magnitude of this risk, however, is still not known, but for couples concerned it is usually the decisive factor in their preference for amniocentesis over CVS. (Chapter 6.4). The total abortion figure after CVS may globally have stabilized at around 4%, but it is still not clear what proportion of this percentage should be considered 'natural background' risk and what proportion should be seen as procedure-related. We made a considerable effort to follow-up any abortion occurring after CVS as completely as possible which enabled us to assign them to either the 'would-have-happened-anyway' category or to the 'due-to-CVS' group.

In the 2-year study period (CVS: n = 216), 10 abortions occurred in total (= 4.6%)[1]. In our first year this was 2 out of 80 = 2.5% and in the second year 8 out of 136 = 5.9%. Abortion is defined here as any spontaneous termination of pregnancy before 28 weeks of gestation age measured from the last monthly period and others will be able to readjust this abortion rate according to whether they are a follower of the 'classical Dutch' line of thinking (pregnancy ending <16 weeks), a supporter of the WHO definition (fetus <500G, or <22 weeks) or prefer the prevailing Anglo-Saxon limit of 28 weeks. In order to make clear how the above mentioned classification was arrived at (see also discussion below), we will give a short telegram-style case report of each abortion separately (w(ks) = week(s) and d = day(s) for clinical history after sampling; previous history, maternal age, indication for CVS, additional details of sampling, etc., see Table 5.9).

[1] Confidence limits 1.9-7.3%; abortionpercentage is 4.8% if calculated from the number of pregnancies intended to continue (216 – 8 therapeutic abortions, see section 5.1).

CASE 1 (recordno. 71).

Clinical course: Started bleeding vaginally 4 days after uneventful sampling, slowly decreasing in amount for 17 days and thereafter dry for 27 days. Then suddenly recurrent blood loss plus abdominal cramp. On examination umbilical cord lying in vagina (fetal heart still positive), followed by expulsion next day (at 16w+6d). Histopathology of fetus normal (100G = 16w), but placenta showed signs of chronic infection in one small area, possibly at site of sampling. Weekly ultrasound follow-up at our department gave a last BPD of 34 mm = 16w+0d, with a slowly decreasing amount of liquor as the only abnormality.

CASE 2 (recordno. 75).

Clinical course: Started bleeding on day 9 (=11w+3d) after a rather difficult CVS (manipulating bladder filling for proper visualization and 3 cannula passes needed for 37 mg blood-stained villi) On day 13 (12w+0d) still blood pv., but ultrasound check normal. Two days later spontaneous abortion at home (12w+2d). Placenta flushed through toilet (thought 'it had nothing to do with CVS'), but fetus sent for histopathology; 8 cm = 13w, normal. Especially no signs of infection (cervical swab before CVS positive: 'anaerobes', not further specified).

CASE 3 (recordno. 167).

Menstrual age unknown, but echoscopically 9w+5d at first CVS. In 3 insertions 23 mg villi of doubtful quality plus some blood, membrane and decidua obtained. No metaphases present. 2 Weeks later second session, ultrasound = 12w+0d and a 5 cm diameter fibroid clearly visible on posterior wall with greater part of placenta situated behind and above it. In one pass 15 mg poor, peripheral villi and ample amniotic fluid aspirated. No immediate subsequent signs of leaking or bleeding. At the time of phoning the result (again no metaphases) on day 5 post CVS, no problems either. But on day 17 (14w+3d) antenatal check-up by midwife: fetal heart negative (though still no complaints). Evacuation elsewhere. Routine histopathology n.a.d. (This patient also participated in our fetal motility study, see section 6.2).

CASE 4 (recordno. 300).

Easy and uneventful CVS in 10th wk. Started brownish vaginal spotting at 17w+0d, 2 days later followed by ample bright red blood loss. Ultrasound check elsewhere: fetal death approximately 12-14th wk, confirmed by histopathology, but no clue as to the cause. P.S.: in the period between CVS and 17w+0d absolutely no symptoms of impending abortion, but no ultrasound check-up either. Note normal MSAFP at 15w+3d (Table 5.9).

CASE 5 (recordno. 317)

Pregnant after long-term primary and secondary infertility. First CVS done in 10th wk, a difficult affair: 3 passes for 30 mg. poor villi (no metaphases found) in a tense and restless patient. Spotting thereafter. Repeat CVS 1 week later (ultrasound= 11 w+4d, normal, especially no indication for intrauterine haematoma), far easier and in 2 passes 43 mg good villi aspirated. Bright vaginal bleeding during the following days and a spontaneous abortion on day 8 after CVS at 11w+6d menstrual age. Histopathology: 'splitting decidual haemorrhage extending intervillously'. Note MSAFP 5 & 5 and 5 & 7 ng/ml, resp., at CVS (Table 5.9).

CASE 6 (recordno. 333).

Placenta situated 'far away' but otherwise uneventful CVS in 10th wk, 85 mg. clean villi in 3 passes. Normal routine 18th wk ultrasound. However, spontaneous abortion at 21w+1d, clinically due to cervical incompetence. P.S.: first pregnancy ended in stillbirth at 29 wks: 69,XXX.

CASE 7 (recordno. 385).

Easy CVS. Fetal death detected in 17th wk. Induced: cord tight around leg. Histopathology: 'occlusion umbilical cord'. See photographs Figure 5.4 a, b and c. (16th wk MSAFP raised).

CASE 8 (recordno. 399).

Easy CVS. Referred by general practioner to obstetrician elsewhere for fetal heart negative. At ultrasound fetal death (12-13 wks). Histopathology: multiple congenital abnormalities i.a. meningomyelocele. Note again normal MSAFP 2 and 4 at CVS, and 5 ng/ml at 16 wks.

CASE 9 (recordno. 405).

Easy sampling at 9w+6d (ultrasound= 8w+6d). No complaints but routine ultrasound in 17th wk no fetal heart. Evacuation elsewhere. Histopathology: 'products of conception with regressive villi changes' (no specific details on dating of fetal parts, haematoma or possible infection).

CASE 10 (recordnr. 418).

Easy CVS and no problems till vaginal bleeding at 21 wks, accompanied by loss of liquor at 24 wk. Delivery at 25w+1d, assisted breech, 830G, surviving >2 years).

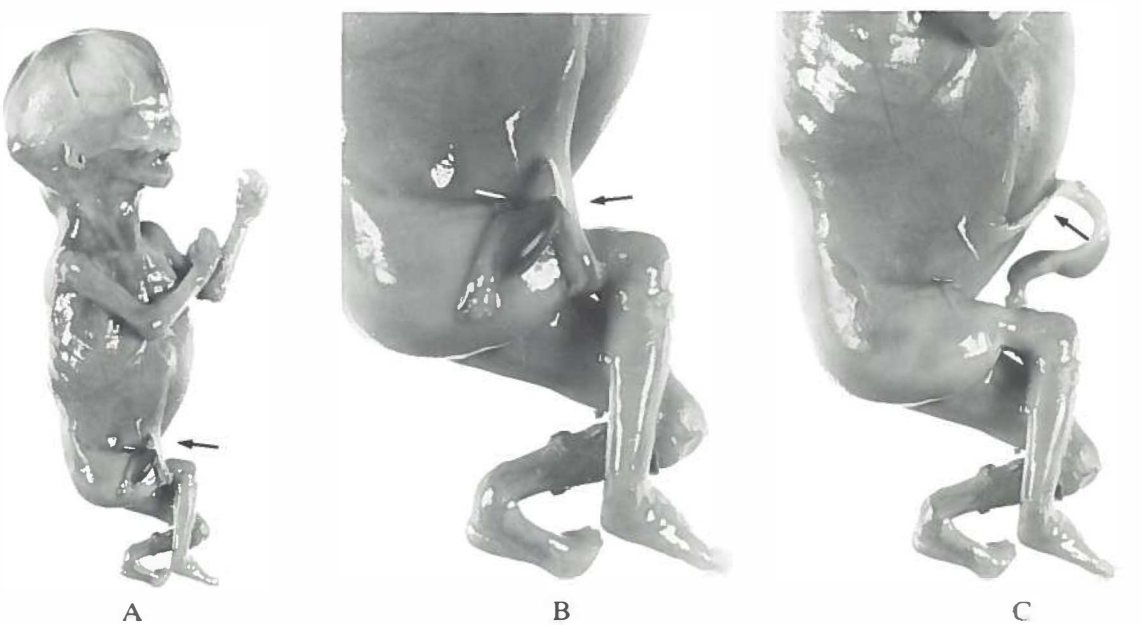


Figure 5.4 Fetal death in the 17th week from umbilical cord occlusion; chorionic villus sampling had been performed 2 months earlier and no subsequent complaints had occurred: A. total view, B. cord around upper leg, C. cord unwound. (case no. 7).
(Department of Pathology, University Groningen)

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	Further details
1	33	1xtri21 1xtri1	0	4	2	1	0	0	0	10+1	28= 9+3	A	I	I	0	55	2	74	12	0	46,XX	10/23	0	16+6	56	–
2	37	mat.age	0	2	1	1	0	0	0	10+1	31= 9+5	A	P	–	0	65	3	37	1(2)	blad manipul.	46,XX	7/12	+	12+2	–	–
3	40	mat.age	0	4	3	0	0	0	0	?	30= 9+5 55=12+0	A A	F F	F F	0 fibr.	95 90	3 1	23 15	1234 15	far away fail	fail fail	nd nd	0 0	15th –	– –	–	Fibroid in the way
4	38	mat.age	0	3	2	0	0	0	0	9+3	37=10+3	A	A	A	0	95	1	28	1	0	46,XY	3/ 3	0	17+2	55	–
5	38	mat.age	0	2	1	0	0	in-fer-til.	0	9+4 10+4	35=10+1 50=11+4	A A	A A	A A	0 0	80 85	3 2	30 43	1 1	pain 0	fail 46,XY	5/ 5 5/ 7	0 –	– 11+6	– –	Tense Easier
6	23	prior 69,XXX	DS NTD	2	1	0	0	0	0	9+4	28= 9+3	A	A	F	0	90	3	85	1	far away	46,XX	4/34	0	21+1	13	Cervical incompet.
7	37	mat.age	0	3	2	0	0	0	0	?	46=11+1	A	A	A	0	80	1	59	1	0	46,XY	13/33	0	17th	152	Cordoccl.
8	37	mat.age	MCA child pater 1st marriage	3	2	0	0	0	0	10+1	26= 9+2	A	A	A	0	40	2	20	1	0	46,XX	2/ 4	0	19+4	5	M.C.Abnormalities i.a. m.m.cele
9	29	1xtri21	DS	3	2	0	0	0	0	9+6	23= 8+6	A	A	A	0	100	1	55	1	0	46,XX	6/ 7	0	16+2	50	–
10	35	anxiety	DS	1	0	0	0	0	0	10+1	30= 9+5	A	P	P	0	80	1	20	1	0	46,XX	10/ 6	0	25+1	72	Survived

Ad column M,N and O : A = Anterior, I = Inferior, P = Posterior, F = Fundal, – = not recorded/not seen.
Ad column T (specimen): 1 = villi, 2 = blood, 3 = decidua, 4 = membrane (? chorion), 5 = liquor, () = very small
Ad case 2: cervical swab = +, i.e. 'anaerobes', not further specified.
Ad case 8: prior MCA, i.e. extensive multiple portwine marks.
Ad case 10: 16th week upper limit MSAFP = 81 ng/ml.

Table 5.9 'Spontaneous' abortion after chorionic villus sampling
(DS = Down's syndrome; NTD = neural tube defect; nd = not done).

Column

A = Case number
B = Maternal age in years and months
C = Indication for CVS
D = (other) Congenital abnormalities in family
E = Gravidity
F = Parity
G = Previous spontaneous abortions
H = Termination of pregnancy
I = 'Relevant' problems previous history
J = Bleeding before sampling
K = Menstrual age at sampling in weeks and days
L = Crown-rump length fetus in mm and corresponding weeks and days
M = Uterus position
N = Placenta localisation
O = Umbilical cord insertion
P = Abnormalities at ultrasound during CVS
Q = Distance from external os to samplingsite in mm
R = Number of cannula insertions
S = Weight of villi in mg
T = Specimen, contents of aspirate
U = Problems at sampling, or on ultrasound afterwards
V = Laboratory result
W = Maternal serum alpha-fetoprotein before and after CVS in ng/ml
X = Result cervical culture before sampling
Y = Abortion week gestational age
Z = 16th Week maternal serum alpha-fetoprotein in ng/ml.

Discussion

Viewed against Harlap's life-table analysis (Harlap 1980), given in Table 5.10, one might conclude that any woman having reached week 10 gestational age (the average week of CVS) still has a risk for a naturally occurring abortion before 28th week (6.7%), which far exceeds the figure usually quoted as the total abortion risk after CVS of around 4%.

Table 5.10 Of the women under observation in week a, b% will abort before week 28 (Harlap 1980)

a	b%
5	14.4
6	10.1
7	9.1
8	8.1
9	7.4
10	6.7
11	6.0
12	4.8
13	3.9
14	3.2
15	2.8
16	2.4
17	2.1
18	1.8
19	1.6
20	1.4

Note: these figures are 'overall' ones, not 'age-specific'.

The problem is this. Looking again at the abortion rate in our study group after CVS, we can safely say that 3 out of the 10 (=1.4%) were caused by the procedure itself (i.e. case numbers 1, 3 and 5), while 4 (=1.9%) definitely had nothing to do with the test (numbers 6, 7, 8 and 10). About the remaining 3 (= 1.4%) we are not sure (numbers 2, 4 and 9). That gives at best an added procedure-related risk of 1.4% and at worst of 2.8% in this series. In our opinion it would be preferable to stick to the 2.8% figure, thus considering all abortions which occur after CVS to be caused by the procedure unless/until proven otherwise and avoiding creation of unfounded optimism.

Are Harlap's calculations too gloomy then? His figures seem sound enough: they were based on no less than 32,182 pregnant women. In our opinion his data are a good estimation of the naturally occurring abortion risk. Nevertheless, they have to be 'corrected' to suit our purpose in order to approximate the added risk of CVS as precisely as possible.

As distinct from Harlap's overall group, our CVS group was preselected on 2 major factors: 'age' and 'normal ultrasound scan'. Therefore the actual question to be answered is: What is the age-specific abortion risk in pregnant women with a normal ultrasound scan at around the 10th gestational week? If

this figure could be assessed, it would be possible to simply subtract this natural background abortion rate from our (also rather age-specific) post-CVS rate, to give us the procedure-related risk. Gustavii (1984) split up the abortion risk in his group of 6,337 women by age and found that for the 35-39 year category 16.7% of all pregnancies came to an end between 7 and 28 weeks. For the 40-plus group this percentage even rose to 33.3%. If we also take a normal ultrasound scan into consideration besides this age factor, the abortion risk is obviously far lower,but even then it increases from 2.6% at the of age 35-39 to 13.6% in women of 40 years and older (Gilmore and McNay 1985).

Kloosterman (1987) carried this a step further. The outcome of his exercise to approximate the age-specific and normal ultrasound (i.e. fetal heart positive) related abortion risk in a group of 1448 women as precisely as possible was 1.9% for women aged 21 - 25, 3.3% for women aged 26 - 30, 2.5% for women aged 31 - 35 and 3.1% for women aged 36 - 40. The number of women over 40 years was too small (n=9) for a meaningful calculation. The average risk for the group as a whole was 2.6% (1.9% - 3.1%). When he took into consideration not only a positive fetal heart but also the echoscopical crown-rump length (CRL), his figures even decreased to below the 1% level, with hardly any difference between the various age-groups.

Now turning to Harlap's table again, it is evident that on the one hand his figures have to be adjusted to fit our purpose, on the other hand they provide a useful basis from which to calculate an approximate risk for abortion naturally occurring within a given period after CVS. If this period is e.g. set at 2 weeks (and abortions occurring within this period are likely to have been caused by CVS), then a spontaneous abortion risk for such a 2-week period after week 9 can be put at 1.5% (7.4 - 6.0 = 1.4 + 0.1 correction) (Huisjes and Mantingh 1984). In associaton with a normal ultrasound scan it will be slightly lower; for older women and a longer than 2-week period it will be slightly higher.

All these figures point in the same direction: the number of abortions that would have occurred after CVS anyway, is far lower than is generally believed. The conditions to be fulfilled before CVS takes place reduce the natural background abortion risk in such a CVS group to not much more than 1.5%. So, if we have a world-wide average abortion rate of around 4% occurring after CVS, then approximately 4% - 1.5% = 2.5% of these abortions must be procedure-related and our figure (between 1.4% and 2.8%) is of the same order of magnitude. Whether this actually means that CVS is consequently 5 times more risky as amniocentesis (usually quoted ad carrying a 0.5% added risk) remains to be seen and is discussed in section 5.2.2.

Given an approximate 2.5% procedure-related risk, is there any chance of decreasing it in future? Not in our opinion, considering the kind of abortions we observed:

Cases 3 and 5 are the debatable ones. Case 3 on account of the sizeable fibroid hampering easy passage of the cannula and acting as a kind of 'ski-jump'. The couple, however, after having been informed about the ultrasound finding at the 2nd CVS session, did not insist but certainly gave us the impression that

they preferred to proceed instead of postponing further examination until 16th week amniocentesis. The abortion in case 5 could possibly also have been avoided by not performing a second session cvs after the first one had failed (N.B. 9 out of our 12 second session CVS were successful). This patient also gave us the impression that she wanted a second try, although she later bitterly complained that we had talked her into it. (As a general rule we do not advocate second tries. However, a substantial number of women specifically asked for CVS because for them 2nd trimester amniocentesis was an absolutely unacceptable alternative and, provided that the echoscopical findings at the 2nd session were normal, we saw no reason to object).

Unfortunately we must conclude that the reality of day-to-day CVS practice dictates a procedure-related abortion risk of approximately 2.5% and prospective patients should be counselled accordingly. In view of Jahoda's finding of a 2.6% post-CVS abortion rate for women of <36 years and 7.2% in women ≥36 years, this 2.5% might even be a conservative estimate (Jahoda et al 1987).

Regarding the other factors such as 'bleeding', 'amniotic fluid leakage', and 'infection' as a cause for abortion:

'Bleeding' (discussed in the previous section) was initially thought to be the foremost indicator of abortion. Alhough decidual haemorrhage easily occurs at sampling, only 2 abortions occurred in a group of 35 women who complained of vaginal blood loss within one week after CVS (the third one started bleeding after 9 days and the remaining 7 women who had spontaneous abortions had no complaints until just before the abortion). Post-CVS bleeding is not comparable with spontaneously occurring blood loss in pregnancy for predicting an abortion. It seems that only the 'abruptio-type haemorrhage' (example case 5) leads to an abortion, the 'extra-placental type', however prolonged and heavy the vaginal bleeding may be, obviously does not (see Figures 5.2 and 5.3).

'Amniotic fluid leakage' (abortion case no. 2) was evidently due to the puncture of both chorion and amnion (at around 10 weeks of pregnancy these are still separated) and subsequent aspiration of a few milliliters of amniotic fluid. Alhough this patient never noticed the loss of liquor after CVS herself, we clearly observed the amniotic sac shrinking slowly during the first half hour after sampling (she was also one of our motility study patients, see section 6.2) and we hoped for the best. In one other patient we most likely also accidently aspirated liquor, but she had an uneventful pregnancy thereafter and delivered a healthy full-term infant. The impression is that one really has to do one's best to puncture the gestational sac with a rather blunt cannula, probably because the mucus filled extra-embryonic coeloom acts as a kind of protecting shield. However, in both cases we were unpleasantly surprised at how easy it was. From the early Chinese reports (Anshan 1975) we know that the puncture of an amniotic sac early in gestational life, is compatible with normal pregnancy outcome in many cases.

As regards 'infection': in case no. 1 it is likely that infection caused the abortion in the 17th week (7 weeks after CVS) It fits perfectly the entity 'early

chronic oligohydramnios syndrome' that was stipulated as a cause for rather late, yet procedure-related abortions (as opposed to a clinically frank infection immediately following CVS), and has been described in one of Jackson's CVS News-letters (1987/21):

> *'Two principal concerns of the CVS investigation from its outset have been bleeding and infection. Although neither has apparently threatened the mother except in isolated instances, there is evidence that some combinations of the two may play a role in leading to loss of the fetus through spontaneous abortion. That this should be so is interesting since these same factors are suspected of playing a principal role in the risk of fetal loss after amniocentesis. The association of bleeding followed by signs of amniotic volume decrease is documented for one of our cases as follows: A 38-year-old patient had profuse bleeding immediately after withdrawal of the catheter which took some time to subside. The patient had light to moderate bleeding following this which continued for 1-2 weeks. This bleeding mixed some bright and apparently fresh blood with darker, discolored material. No clinical evidence of infection or amniotic fluid leakage was noted. This discharge persisted intermittently over several weeks. At 16 weeks gestation, this was accompanied by signs of oligohydramnios and predicted threat of fetal loss. Several ultrasound examinations were done during this period and none showed evidence of damage to the membranes or the formation of a hematoma. With the persistence of fluid diminution through unproven leakage or decline in formation, the patient and the attending physician elected interruption of the pregnancy'.*

For other seemingly obvious factors, such as 'number of cannula insertions', (e.g. in 20 patients more than 2 cannula insertions and 2 abortions in this group: at 12 wks in case 3 at 21 wks in case 6, see section 5.1.4.2) 'total weight of villi' sampled, 'kind of specimen' (only villi or accompanied by blood, decidua, membrane-like tissue, etc.), 'intrauterine haematoma after CVS at ultrasound', or a 'positive cervical swab' before sampling, we could not indicate a specific association with CVS-abortion. The results of our maternal serum alpha-fetoprotein measurements before and after CVS as well as at 16 weeks and their significance are dealt with in section 6.1.

The photographs shown in Figure 5.4 are intended to act as a reminder that inspection of the products of conception with the naked eye can often exonerate CVS from the blame of having caused the abortion and relieve the patient from feelings of guilt which are invariably present in such circumstances.

5.1.4.3 Abdominal discomfort

In line with Jackson's registry form, abdominal discomfort after chorionic villus sampling (CVS) was marked as a separate item in our check-list. In practice, however, only three women mentioned it spontaneously as a sole, distinct complaint that persisted and had worried them for a few days after CVS[1]. All three delivered a healthy infant at 34w+3d, 41w+3d and 41w+3d respectively. When other women were specifically asked about this kind of problem, many of them said that they had experienced something of the sort for some time, but had considered it to be normal and nothing to worry about.

[1] Pain in association with vaginal bleeding is excluded.

5.1.4.4 Infection

For a while we contemplated the relevance of including this chapter, considering it too much of an exercise in futility in view of the single case of an abortion possibly caused by an infection (case no. 1, pre-CVS smear negative, section 5.1.4.2). In general terms, however, the infection issue is still very much under discussion and makes a considerable impact on e.g. whether to prefer the abdominal 'germ-free' needle technique, or the potentially contaminated transcervical cannula approach. Moreover, some frightening case histories have been described (Jackson 1985/10, 1987/23; Muggah 1987). Other infection-related topics such as whether an intrauterine contraceptive device in situ forms a contraindication for CVS, or whether women should be treated prophylactically in the case of a positive cervical smear at intake, also keep our attention drawn to this issue. Long-term effects such as late intrauterine fetal death, preterm labour or rupture of the membranes, intrauterine growth retardation, or neonatal infection, could also possibly be the result of bacterial invasion of the uterine cavity (Brambati and Varotto 1985).

Cervical swabs were taken routinely just prior to CVS and sent to the 'Streeklaboratorium voor de Volksgezondheid' (Regional laboratory for Public Health), Groningen, for routine investigation for 'pathogenic micro-organisms'. The test was positive in 19 out of 196 CVS cases (10.6%)[1]. No therapy was given. Table 5.11 gives an overview of the pregnancy outcome in these 19 women.
In our study group (n=427), 6 women complained at intake of vaginal discharge, 3 are mentioned above, in 2 the cervical smear was negative, and in one a smear was not taken. The role 'infection' played in the drop-out group is described in sections 3.2 and 5.2.2).

Discussion

The infection issue hovers between two extremes: on the one hand '*CVS should not be accepted as a routine method of prenatal diagnosis until microbiological investigation has confirmed that it does not increase fetal and maternal morbidity*' (Gardner et al 1985) and on the other hand '*we did not find any significant clinical infection in more than 400 patients and conclude that proper disinfection before CVS is a successful preventative measure*' (Holzgreve et al 1986).

Actually, the discussion about infection-related CVS problems was sparked off by the findings of Kullander and Sandahl (1973). They did not conduct follow-up research on CVS when they encountered two complications of gonococcal amnionitis after transcervical sampling with their endocervicoscope, as we saw in section 2.1.2.

Then Jackson described three cases of post-CVS infection in his News-letter of April 1985 (no. 10) reported to him by three different centres in the USA:

[1] The 9 Arnhem cases and 11 others in whom no smear was taken were subtracted from our n=216 CVS group.

Table 5.11 Pregnancy outcome in 19 CVS cases with a positive cervical smear, without treatment.
(D = vaginal discharge before CVS; Cx = cercical smear; PIH = pregnancy-induced hypertension.
See list below table for explanation of numbers; other abbreviations are self-explanatory).

Recordno.	D	Cx	Fetal loss	Week	Weight	Problems
69	–	1	–	38	2765G	PIH + catapresan
75	–	8	Sp.Ab (12+2)			
100	–	1	–	38	3380G	–
110	+	6	–	39	3240G	–
116	–	1	–	38	3530G	–
125	–	+–	–	34	2660G	Preterm delivery eci
127	+	7	–	35	2250G	Prem. rupture membranes eci
166	–	5	–	39	3880G	–
168	–	5	–	41	3500G	–
194	–	1	–	38	3020G	–
277	–	+–	–	40	3550G	–
280	–	1	–	39	3720G	–
192	–	1	–	40	3800G	–
297	–	1	–	39	3750G	–
321	+	1	–	40	4010G	–
379	–	5	–	40	3450G	–
408	–	5	–	37	2850G	Vaginal bloodloss 13w
409	–	1	Ind.Ab (tri.21)			
423	–	1	–	40	3280G	–

0 = 'no pathogenic micro-organisms' or 'sterile'
1 = *candida albicans*
2 = *trichomonas vaginalis*
4 = *E. coli*
5 = β haemolytic streptococci
6 = enterococci
7 = *Gardnerella vaginalis*
8 = 'anaerobes'
+– = gram-positive cocci seen, but no growth

clinically insiduously starting, but suddenly progressing to septic shock either with or without disseminated intravascular coagulopathy; a picture reminiscent of IUD-related infection.

In June 1985 Gardner et al followed with a report of their findings in 7 patients who had consented to CVS before termination of pregnancy. Six had a positive cervical smear taken before sampling and in two of them pathogenic micro-organisms were cultured from the catheter. The presence of *Chlamydia trachomatis* in one patient was especially worrying. The long-term clinical significance was not tested as the pregnancies were terminated 24 hours after CVS. Their conclusion is cited in the first paragraph of this section.

Within a few months thereafter, several other reports were published. Wass and Bennett (1985) described 13 patients in whom only *candida vaginalis* was found (and no β-haemolytic streptococci, *Neisseria gonorrhoeae* or *chlamydia*

trachomatis!) and whose pregnancies progressed normally after they had been treated before undergoing CVS.

Blakemore et al (1985) reported a case (one of Jackson's) of post-CVS uterine infection initially slowly brewing but on day 12 suddenly developing into a full blown septic shock, treated by evacuation on day 13, followed by an uneventful recovery. A cervical swab was not taken before CVS but a cervical culture performed before evacuation showed *Bacteroides fragilis*. Sampling itself had been unremarkable except for two passes using the same catheter.

Brambati and Varotto (1985), however, did take cervical swabs at intake, some two weeks before CVS. Despite treatment, some 20% of their first 560 cases still had a positive cervical culture, either the same or quite different micro-organisms being found. In their first 700 diagnostic CVS procedures they saw 2 infections, also with septicaemic fever appearing 2 weeks later followed by foul vaginal discharge and abortion. In both cases CVS was uneventful with only one pass of the cannula. *Gardnerella vaginalis* was seen in one smear taken at sampling, the other swab was negative. The products of conception were not cultured. They estimated the risk of acute infection after CVS to be 3 per 1000 and drew attention to the possibility of long-term effects like the ones mentioned in the introduction above, stating that there is simply not enough information available on organisms which are potentially dangerous and about the clinical significance of a 'normal' vaginal flora.

McFadyen et al (1985) concluded from their microbiological findings in a group of 49 patients who underwent termination of pregnancy and in another group of 14 women who underwent CVS, that the presence of cervical mycoplasmas and *Gardnerella vaginalis* was insufficient reason for prophylactic antibiotics, but they also advised that CVS should be done onder antibiotic coverage if micro-organisms 'of greater potential pathogenicity' were found at intake.

Later Muggah et al (1987) described a patient who developed septic endotoxic shock 28 hours after uneventful amniocentesis that was preceded by two unsuccessful CVS procedures a few weeks earlier (3 cannula passes in total, cervical smears not mentioned). A hysterotomy was done; fetus and placenta were clearly infected (*E.coli*!), although the amniotic fluid was sterile. They suggested that the amniocentesis might have simply exposed an already subclinically present intrauterine infection.

In general, our findings are similar to the above experience. We had one late abortion possibly caused by an infection (with a negative smear pre-CVS and after an uneventful sampling with 2 passes) in a total group of 216 diagnostic cases. There was no evidence of an increased risk for preterm labour, growth-retardation, neonatal infection, or fetal anomaly in the 19 women who had a positive smear before CVS.

By the time of writing this thesis , the Dutch Working Party on Prenatal Diagnosis reached consensus on an issue based on the evidence accumulated at all antenatal diagnostic centres in the Netherlands: the need for a routine pre-CVS cervical smear in (discharge-free) women was found to be unnecessary as it all too often results in information we do not know what to do with.

The infection risk will probably remain inherent to the procedure itself, regardless of the cervical culture being positive or not, analogous to second trimester amniocentesis. It means that the conclusions of Leschot et al who relativized remarks about CVS propagated as being 'quick, safe and reliable' (Galjaard 1985), still stand (Leschot 1986, Kloosterman and Christiaens 1986, Bennebroek Gravenhorst 1986). The infection factor remains an important determinant for the safety of CVS. Whether women with a positive smear at intake

- should be treated with an antibiotic and the CVS postponed until a negative swab is obtained, or
- should be treated with an antibiotic prior to CVS with no follow-up swab, or
- should undergo CVS under antibiotic cover, or
- be offered transabdominal CVS or amniocentesis,

as put forward by Wass and Bennett (1985), is not clear. For the time being we have not progressed further than to advise working 'antiseptically' and using a fresh cannula for every additional pass.

5.1.4.5 Amnotic fluid leakage

Amniotic fluid leakage was also marked as a separate item on our check-list, again in line with Jackson's registry form, as was the case with 'abdominal discomfort' mentioned in section 5.1.4.3. However, nobody in our group complained of leakage of liquor after CVS. Not even in the two women in whom we had every reason to believe that we had punctured their amniotic sac at sampling, followed by aspiration of at least a few milliliters of liquor. One of them had an abortion 2 - 3 weeks after CVS without having had any evidence of amniotic fluid leaking during this period. This case is described in section 5.1.4.2. The other one was a 36-year-old para 4 with a clean previous history (except for her third child who had exomphalos and exstrophia vesicae). CVS was done for advanced maternal age at 12 weeks + 6 days (echoscopically, the last monthly period being unreliable), with an anterior placenta in an anteverted uterus. Under poor visualization, 30 mg of poor villi were obtained in the first pass using a Portex cannula. During the second attempt some fluid was clearly aspirated while sweeping the placenta with the cannula. The ultrasound check was normal and she delivered a healthy 3000G boy in the 38th week after an uneventful pregnancy. (From the outset she had declined amniocentesis, which would have been the method of choice visa her previous history).

In view of the large, mucus filled extraembryonic coeloom still present between the amnion and the chorion at the usual time of CVS, around the 10th week of gestation, it seems almost impossible to perforate the amnion with the blunt Portex cannula. Often indentation of the sac can clearly be seen echoscopically during sampling, seemingly without any harm being done. We will leave these two casuistic experiences for what they are and not enter into further discussion apart from mentioning the early Chinese experience (Han Anguo et al 1985) where aspirations of amniotic fluid were relatively common (because of the 'blind' technique being employed), but did not usually give rise to any problems later on.

5.1.4.6 Anti-D anaphylactic shock

One case history is presented here to illustrate first, that giving anti-D gammaglobulin (anti-D) to Rhesus negative women prophylactically after CVS because it cannot do any harm and it may do some good is inappropriate and secondly to lend support to section 6.1, which aims at, inter alia, calculating the minimum amount of anti-D required post-CVS.

The lady in question was a 38-year-old Rhesus negative para 2, who underwent CVS for advanced maternal age. Sampling at 9w+4d was somewhat difficult because the placenta was situated partly over and above a posterior wall fibroid (appr. 5x4 cm). In 2 aspirations 78 mg of good quality, blood-stained villi were obtained. A small haematoma was seen on the ultrasound scan directly after sampling (see picture Figure 5.1). Half an hour later, after she had been given 200 microgram anti-D intramuscularly, the hospital police informed us that she had just collapsed at the hospital's exit gate. She was admitted in anaphylactic shock but recovered quickly after appropriate treatment. Apart from a 'non-specific allergic constitution', no particular explanation could be found despite extensive laboratory follow-up. On 2 previous occasions she had received anti-D without the slightest evidence of any allergic reaction to it.

This is not the place for an extensive discussion on the benefits and dangers of anti-D prophylaxis. For further information we refer the reader to Mollison et al (1968), Bennebroek Gravenhorst et al (1984), and Tabsh et al (1984). Especially the latter authors have dealt with the harmful aspects of anti-D immunoprophylaxis after antenatal diagnosis.

5.1.5 Long-term follow-up (≥28 weeks of gestation)

5.1.5.1 Pregnancy complications

One might wonder what purpose is served by a description of pregnancy problems in a group of women who underwent chorionic villus sampling (CVS) if a proper (a-select) control group for comparison is lacking. As randomization had not been applied we settled for comparing our 'CVS group' (A) with the 'amniocentesis group' (B) and the 'neither CVS nor amniocentesis group' (C) from the CVS study group (n=427), in order to obtain a clinical impression of whether complications differ widely, or whether it is likely that having undergone CVS or not makes any difference in terms of pregnancy complications and fetal outcome. Table 5.12 below gives an overview of pregnancy problems from 28 weeks onwards in these 3 groups, without further discussion.

5.1.5.2 Pregnancy Outcome

In the post-CVS pregnancies intended to continue to term, two children were born with congenital abnormalities: one Fallot's tetralogy (preterm, 25 percentile) and one cheilognathopalatoschizis (small for date, 5 percentile).

Table 5.12 Course of pregnancy after chorionic villus sampling (CVS), amniocentesis (AC), or after neither CVS nor AC.
(n = number of pregancies intended to continue to term; explanation of abbreviations below table)

	A	B	C
	CVS	AC	no CVS/no AC
	n=206[1]	n=127[2]	n=27
SFD[3] only <10 p	2	3	3
< 5 p	3	2	
< 2.3p	3	4	1 (twin)
Preterm delivery (<37wks):			
- after ROM + AFD	34+6 5 35+0 35+0 35+4 36+1	29+4 5 31+4 34+6 35+4 36+1	31 1
- otherwise (APH, PIH and AFD)	34+3 5 35+3 36+2 36+5 36+5	32+4 5 34+4 35th 35+5 36th	
(average gestational week:	35+4	34+5)	
- after ROM + SFD	0	0 0	
- otherwise + SFD (2.3-5p)	34+4 1	35+6 1	0
Premature ROM without premature delivery	1	0 0	
Total	20 (=9.7%)	20 (=15.8%)	6
A few other possibly CVS-related problems:			
APH	5	5	1
Premature contractions (only)	6	2	0
PIH - admitted	5	5	1
PIH - ambulant	3	5	1
Total	19 (=9.2%)	17 (=13.4%)	3

p = percentile
SFD = small-for-date
ROM = rupture of mambranes
AFD = appropriate-for-date
APH = antepartum haemorrhage
PIH = pregnancy-induced hypertension

[1] Excluding one delivery <28wks (see section 5.1.4.2).
[2] Excluding 2 deliveries <28wks (see section 5.2.2).
[3] Excluding infants with congenital malformations (see next session).

Another infant, who appeared to be 'dysmorphic' at birth (39w+2d and 1950G, <2.3p) has caught up and is doing well now. Also a concerned mother asked whether a persistent bald spot on her 1-year-old boy's head could possibly have been caused by CVS.

Stillbirths, neonatal deaths and maternal deaths did not occur. The general delivery profile of our group in terms of duration of gestation, mode and place of delivery, weight, etc. did not differ from the overall delivery performance of the total group of women giving birth at our clinic. This is remarkable taking into consideration that our CVS group was biased towards e.g. older age and fertility problems.

5.2 Results of 211 'drop-outs'

5.2.1 Abortion

An overview of the reasons why 211 women 'dropped-out' of the total chorionic villus sampling (CVS) intake group (n=427), was given above (section 3.2), the main groups of reasons being:

- Woman expressed personal preference for amniocentesis
- Had a spontaneous abortion
- Was advised amniocentesis by us
- Woman changed her mind altogether and had neither CVS nor amniocentesis, or preferred termination of pregnancy.

Sixty-six abortions occurred either between application for CVS and intake, or even between intake and scheduled CVS, i.e. 13.3% of our study group of 427 women[1]. Table 5.13 categorizes them in A. spontaneous abortions and B. terminations of pregnancy.

Particulars of these 4 subgroups are given in Table 5.14, with the abbreviations explained at the end of the list.

[1] Subtracting the 10 TOP's (n=10), 15.5% becomes 13.3%.

Table 5.13 Overview of abortions before chorionic villus sampling (TOP=termination of pregnancy)

n=427	Before intake	After intake	Total
	I	II	
A. Spontaneous abortions	13	43	56
B. TOP	4	6	10

Table 5.14 Particulars of abortions occurring before chorionic villus sampling (abbreviations at bottom of list)

Group A I: Abortion after application for CVS and before intake, according to maternal age (n=13)

Maternal age in years	Abortion in weeks + days
27	6+6
29	*
30	8
36	10
36	*
36	10+6
37	9
38	*
39	10
40	8
40	9+4
42	*
44	*

Group A II: Abortion after intake for CVS, according to menstrual age at intake (n=43)

Maternal age in years	Menstrual age at intake in weeks + days	US at intake in mm and corresponding gestational age	Fetal heart pos.or neg.	Abortion in weeks and days
37	7+3	CRL 13 = 7w+3d	P	*
40	7+4	sac 16 = 5w+5d	–	*
40	7+5	sac ?diameter	–	9+ 2
38	8+0	–	–	9+0
37	8+0	CRL 11 = 7w+1d	P	10
37	8+0	CRL 14 = 7w+5d	P	10
40	8+1	CRL 11 = 7w+1d	P	9+5
36	8+1	sac 33 = 8w+1d	–	10+0
42	8+2	CRL 7 = 6w+2d	P	10+6
39	8+2	sac 21 = 6w+3d	–	9+6
38	8+2	sac 33 = 8w+1d	–	10+2
38	8+2	sac 21 = 6w+3d	–	9
38	8+3	CRL 3 = 5w	N	9+0
37	8+3	sac 36 = 8w+3d	–	10+2
38	8+4	sac 21 = 6w+3d	–	9+4
36	8+5	CRL 11 = 7w+1d	N	11+2
39	8+5	–	–	*
39	8+5	sac 13 = 5w+2d	–	9+4
37	8+5	CRL 21 = 8w+4d	N	9+6
P=62	8+6	CRL 10 = 7w+0d	P	10
42	9+0	sac 36 = 8w+3d	–	12+0
32	9+0	CRL 17 = 8w+1d	N	10
41	9+2	–	–	*
40	9+2	CRL 24 = 9w+0d	N	9+6
39	9+3	CRL 12 = 7w+2d	P	12
41	9+3	sac 35 = 8w+2d	–	10
34	9+4	–	–	9+6
37	9+4	CRL 16 = 8w+0d	?	10+2
40	9+4	CRL 15 = 7w+6d	P	14
36	9+4	CRL 23 = 8w+6d	?	10+6
36	9+4	sac 51 = 10w+	–	10
38	9+5	sac 12 = 5w+1d	–	10
41	9+6	CRL 13 = 7w+3d	N	11
39	9+6	CRL 12 = 7w+2d	N	9
27	10	–	–11	
40	10+0	sac 18 = 6w+0d	–	12+0
38	10+2	–	–	10+6
38	10+2	CRL 16 = 8w+0d	N	11+2
29	10+4	CRL 5 = 5w+6d	N	10+
38	10+5	–	–	10+5
37	10+5	CRL 4 = 5w+	?	*
35	13+0	sac 18 = 6w+0d	–	14
41	?	sac ?diameter	–	*

Group B I and B II: Induced terminations of pregnancy after application, before intake for CVS (B I; n=4) and after intake (B II; n=6), in chronological order.

No	Maternal age in years	Menstrual age at intake in weeks + days	G	P	A	TOP	Additional problems and days	Abortion in weeks
B I:	44	–	–	–	–	–	–	
	40	–	–	–	–	–	SIDS, d.mell.[2]	–
	41	–	–	–	–	–	–	–
	38	–	–	–	–	–	–	9
B II:	39	8+3	3	2	0	0	Hypothyroidism	9+4
	38	8+1	5	1	1	2	–	–
	41	8+1	4	3	1	0	Twin <28w, died	9
	39	10+1	5	3	1	0	Besnier Boeck	–
	37	9+4	3	2	0	0	–	10
(After CVS:								
10.	37	9+5 4 3 0 0					Adnexectomy	10+6[3] (granulosa-celltumor)

Abbreviations used:

*	= 1st trimester abortion, but exact date not known
US	= ultrasound
mm	= millimeters
FH	= fetal heart
CRL	= crown-rump length in mm + corresponding gestational age
sac	= gestational sac in millimeters diameter
P	= positive
N	= negative
P=62	= pater is 62 years old
?	= 'something present', but not convincingly
TOP	= termination of pregnancy
G	= gravidity (previous number of pregancies)
P	= parity (previous number of deliveries)
A	= abortion (previous number of spontaneous abortions)
TOP	= termination of pregnancy
SIDS	= sudden infant death syndrome
–	= not relevant, not mentioned, or not available.

[2] Admitted elsewhere for regulation of blood sugar. Was advised to undergo amniocentesis instead of CVS in view of diabetes mellitus. Patient then changed mind in due course and had TOP in hospital for emotional reasons.

[3] Had undergone TOP elsewhere the day after the normal result of CVS (46,XX) had been given by phone. Sex was not mentioned; she had 3 boys.

Discussion

Except for case no. 42, all abortions were 1st trimester (in an approximately 50:50 ratio 'blighted':'missed'). This was Gustavii's (1984) main reason for waiting until this period had passed and performing CVS at the beginning of the second trimester. Crown-rump length (CRL) measurements in this group ranged from 3 to 24 mm CRL (5w - 9w+0d), the ones measuring >15 mm all had fetal heart negative. In combination with a positive fetal heart, CRL measurements ranged from 7 to 15 mm. Viewed the other way around: a positive fetal heart, together with a CRL of >15 mm (7w+6d) was never associated with a spontaneous abortion in our total group of 427 CVS. Although we are dealing with a population of women who were heavily biased for age, this finding is in line with our discussion in section 5.1.4.2 on natural background versus CVS-related abortion risk and corresponds well with Kloosterman's (1987) results. He arrived at a CRL figure of 20 mm (8w+4d), after which differences in maternal age did not matter anymore.

It was also remarkable that of the 43 women who had a spontaneous abortion, only 13 of them (30%) complained of vaginal blood loss at intake (7x fresh bright, 5x old brownish and 1x watery pink discharge).

Another issue regarding future CVS or amniocentesis concerns the degree of importance which should be attached to a non-confirming ultrasound result: in our study group (n=427) we had 12 women at intake with either a negative discongruency, negative fetal heart, or both, who were later found to carry viable pregnancies and subsequently underwent successful CVS or amniocentesis, see Table 5.15.

Table 5.15 Viable pregnancies despite poorly corresponding ultrasound findings at intake for CVS, in chronological order (n=12)
(CRL = crown-rump length; AC = amniocentesis; N = negative)

Menstrual age in weeks and days at intake	CRL/sac in mm	Fetal heart	CVS/AC
9+2	CRL 27 = 9w+2d	?	CVS
7+4	–	–	AC
7+3	sac 35 = 8w+2d	–	CVS
9+3	CRL 10 = 7w+0d	N	AC
9	–	–	AC
7+3	CRL 12 = 7w+2d	N	AC
8+4	CRL 13 = 7w+3d	N	AC
8+3	CRL 9 = 6w+5d	N	AC
10+6	CRL 15 = 7w+6d	N	AC
9	sac 13 = 5w+2d	–	CVS
8+5	sac 20 = 6w+2d	–	CVS
8+0	sac 24 = 7w+0d	–	AC

Again, with a CRL of ≤15 mm things can still go either way, but it becomes a different matter when the CRL is >15 mm.

Obviously the increased age-related spontaneous abortion risk is caused by the increased frequency of chromosomally abnormal fetuses. However, by the

time an embryo has reached a certain size (15-20 mm), it is attractive to assume that the chromosome factor hardly plays a role anymore and the remaining spontaneous abortion risk thereafter drops to a non-maternal age-related level (see also section 5.1.4.2).

Ten women preferred to undergo termination of pregnancy after all: 4 after application but before intake, 5 after a normal intake and one even after successful sampling had been performed. This confirms our impression that for some women the length of time between a positive pregnancy test and the first arrangements for CVS is too short to make up their mind.

This 'abortion group', comprising spontaneous abortions often just evident at intake and women who prefer an induced abortion after all, makes a major contribution to the total drop-out group and is not expected to change in size in the future. As far as CVS is concerned, the time and effort spent per patient during intake and sampling already compares unfavourably with 2nd trimester amniocentesis and, in our experience, part of it will turn out to have been 'wasted' anyway (Mantingh et al 1986a).

5.2.2 Amniocentesis and follow-up

An overview of the reasons for dropping-out is given in chapter 3.2. In terms of total figures, the reasons that led to the women undergoing amniocentesis were the most important ones. We discussed the runner-up, i.e.'abortion', in the previous section. This chapter deals with amniocenteses: n=135[1] (=31.6% of total n=427 study group). This is our total amniocentesis figure, made up of 117 women (27.4%) whose personal preference was amniocentesis (I), women who were advised by us to do so (II) and by those who needed confirmatory amniocentesis (AC) for a metabolic disorder, ambiguous karyotype or failed CVS (18 in total, 4.2%), expressed in numbers as follows:

– women who preferred amniocentesis themselves (I)	69	> 117
– were advised by us to undergo amniocentesis (II)	48	
– failed CVS	11[2]	
– confirmatory AC for metabolic disorder	5	
– confirmatory AC for ambiguous karyotype	2	
	135	

It is the n=117 group (I + II) we are going to deal with here.

[1] Excluding one patient we referred to Rotterdam for Zellweger, who was found to have a positive cervical smear and was advised amniocentesis.

[2] This number is lower than the total number of unsuccessful CVS we performed, as some patients subsequently declined amniocentesis.

As regards group I, we divided the reason(s) the women gave into (one of) the following groups:

1. Amniocentesis carries a smaller abortion risk.
2. Amniocentesis is more complete, it tests for neural tube defects as well.
3. CVS is new and less reliable.
4. There might be other CVS related risks, such as infection, fetal growth retardation, or premature rupture of the membranes.

Looking at the reasons put forward at intake, or later e.g. by phone when a patient changed her mind after all and cancelled her appointment for CVS, 'abortion risk' was mentioned most frequently. It was given as the main by 59 women (=50.4%). 'Incompleteness' was given by another 10 as the main reason (=8.6%).
Of course, other associated reasons were often mentioned as well, but were clearly ranking second or third in importance compared with the two mentioned above. In this way 'incompleteness' was mentioned 11 times as an associated reason, 'new, unreliable and other risks' 5 times and 'sorry, must go on holiday' twice.

The remaining group (II) of 48 women were advised (in varying terms, from mildly putting off to strongly advising against the CVS option) to undergo amniocentesis for various reasons, based on previous history, ultrasound findings at intake, problems during the CVS session, etc. A short description of these reasons is given below; more or less related ones were combined, 'A' to 'J' included.

A. Vaginal bleeding. 8

Women recently having suffered vaginal bleeding (usually within a week of scheduled CVS) or actually still bleeding at the time of intake regardless of whether the intrauterine situation was echoscopically normal or showed explanatory features, such as a second sac or haematoma formation (besides a viable pregnancy, of course: abortions are dealt with in section 5.2.1).

B. Multiple gestations. 6

Record no.
91: Twin at sampling ultrasound (missed at intake).
99: Twin.
163: Second sac and a 53x57 mm. fibroid anterior wall (G4, P1, A1, AAP1).
224: Twin (at amniocentesis 1x trisomy 21, selective fetocide, see below).
226: Twin.
329: During CVS session: second sac situated inferior over internal os. At probing it shifted up ahead of canula, keeping placenta out of reach.

C. Positive discongruency/gestational age (g.a.) too advanced. 9

76: At intake g.a.=9+0 and CRL 12w+5d (pregnant while breast-feeding).
171: At intake g.a.=11+5 and CRL 12w+3d.
173: At intake g.a.=11+0 and CRL 11w+1d (too far and having to wait for health insurance co. to agree with 'anxiety' indication: husband's brother trisomy 21).
180: At intake g.a.=8+1 and CRL 12w+2d
182: At intake g.a.=12+2 and CRL 12w+? (but 'wanted to try').

310: At intake g.a.=9+6 and sac only. At g.a.= 10+6, CRL 7w+6d, poor view, uterus in RVF and FH negative. At g.a.=11+6, CRL 12w+3d
374: At intake g.a.=8+3 and CRL 12w+4d.
392: At intake g.a.=11+1 and CRL 12w+0d.
412: G2, P0, A1, indication 'anxiety', wife's sister was trisomy 21. At intake g.a. =8+0 and only sac seen, 24 mm 7w+0d. By the time health insurance co. agreed to accept this indication, the confirmatory ultrasound elsewhere was acceptable, but too late for CVS.

D. No indication or wrong indication. 2

44: 'Several' neural tube defects in family.
186: Previous anencephalic dead fetus.

E. Chemotherapy or radiotherapy. 2

126: Chemotherapy carcinoma testis (+twin!).
255: Full dose radiotherapy for cancer of spinal cord (maternal)

F. Infections. 2

190: Recurrent genital herpes active at intake.
221: Vaginal discharge +++. Cervical swab at intake: *Gardnerella vaginalis.* Moreover, at g.a=10+5, CRL 11w+4d.

G. Intrauterine Contraceptive Device (IUD) related. 4

108: Pregnant with IUD still in situ (fundal).
150: Pregnant despite IUD, removed at g.a.= 9+0. At g.a.=10+6, CRL 8w+4d. CVS not mentioned in view of this discongruency and last 2 pregnancies having ended in a spontaneous abortion.
281: Pregnant despite IUD, removed. G.a unknown, CRL 11w+2d with a fundal placenta.
351: Pregnant despite IUD in situ (for 12 years), removed at appr. 8 weeks. Two children, 17 and 14 years, and pregnancy initially rejected. Then took risk for being too late for CVS (holiday): at g.a.=11+3, CRL 11w+5d.

H. 'Technical' problems. 7

124: At CVS session visualization too poor, despite uterus in AVF and placenta posterior. Postponed 1 week, then later patient subsequently cancelled in favour of amniocentesis because of CVS-related abortionrisk. Had a 16th week amniocentesis and aborted in 20th week (see below at follow-up).
183: A 30x40mm. fibroid on posterior wall with the placenta behind it. Probing at CVS session: cannula obstructed by fibroid.
192: Consistent poor view of an uncorrectable retroverted uterus with a posterior placenta at sampling session, despite bladder manipulation by varying amounts of normal saline.

200: Same situation as recordno. 183, but probe entered – uterine cavity anteriorly. No CVS attempted.
267: Retroverted uterus with anterior placenta seemingly out of reach with poor view at CVS session.
270: Same situation as recordno. 267 with even worse visualization. Patient (G1, 2nd marriage, and 43 years of age), did not want us to proceed.

369: At CVS session poor view of retroverted uterus and posterior placenta lying at 90° to cervical canal, despite different bladder fillings.

I. 'Assorted' problems i.e. miscellaneous other reasons for us to advise amniocentesis. 8

21: Extra time needed for further investigation as patient with cheilognatopalatoschizis and factor VIII deficiency herself, reported at intake to have several Down syndrome children in her family.
38: Fibroids + on heparin-therapy for thrombotic leg + possibly carrier t(1;2)(q44;q33).
203: Our fault: CRL 41 mm reported as 11w+4d instead of 10w+5d, and subsequently advised for amniocentesis.
265: Advised against CVS as ultrasound showed discongruency between CRL 9 = 6w+5d and diameter sac 60 11w+5d. At follow-up no difference anymore, of course (our mistake too).
296: Gravida 1, aged 38. Primary infertility for many years in previous marriage and again for 2 years with 2nd husband aged 47 years. At probing cervical stenosis, dilatable. yet no further attempt as CRL 11w+3d, cord insertion in fundo and couple no strong preference for CVS.
308: 38-year-old G4, P0 and A3. Had exconisation for carcinoma in situ after which secondary infertility for 3 years. This time pregnant after clomiphene. Phoned us after having read our info: 'no thank you very much'.
331: Grossly overweight with very problematic ultrasound at intake (g.a.=10+4 and CRL 17 8w+ 1d and ?fetal heart).
426: At intake echoscopically hygroma colli suspected. Early amniocentesis (13 wks): 47,XX,+18.

Total 48

(J: The 'overlap' category is classified as italics. 7)

(126: *Chemotherapy* and twin.

156: *Bleeding* and multiple gestation.

163: *Multiple pregnancy* and fibroids.

221: *Infection* and pregnancy too far advanced

281: *IUD* and too far advanced

298: *Assorted* and too far advanced

397: *Bleeding* and multiple gestation)

Abortion after amniocentesis (<28 weeks of gestation)

As we saw above, this amniocentesis group was made up by 2 main subgroups: (I) patient herself preferred amniocentesis over CVS and (II) patient was advised by us to undergo amniocentesis (and the remaining cases comprised failed CVS or the CVS result needed to be confirmed by amniocentesis). Their follow-up is given in Table 5.16.

Table 5.16 Abortion after amniocentesis <28 weeks gestational age.
(AC = amniocentesis, Sp.Ab = spontaneous abortion, PROM = premature rupture of membranes).

Group	Record number	Why AC	Age in years	Induced abortion	Follow-up[1] + weeks of gestation	Details
I	10	Ab.risk	38	–	Sp.Ab,22+3	No problems till day before expulsion
	11	Incomplete	27	–	Sp.Ab,26+2	Bloody tap. Premature contractions. Died
	63	Ab.risk	35	–	Sp.Ab,23+0	No complaints
	87	Ab.risk	36	+	Trisomy 21	
	172	Ab.risk + our advice	36	–	Sp.Ab,25+4	AC no problem. PROM
	197	Ab.risk	37 + incomplete	–	Sp.Ab,16	Traumatic AC
	289	Ab.risk	38	–	Sp.Ab,20	Amniotic fluid leakage after AC
II.	124	Ab.risk	38	–	Sp.Ab,19+1	Suspected intrauterine infection
	190	Herpes genit.	37	–	Sp.Ab,26+2	AC easy. Cervical incompetence. Died
	224	Twin	38	+	–	1x trisomy 21. Selective fetocide
	426	?Hygromaat intake	36	+	–	Trisomy 18
Rest	27	–	34	+	–	Turner's syndrome

[1] For follow-up ≥28 wks. see sections 5.1.5.1 and 5.1.5.2.

Discussion

Eight 'spontaneous' abortions at <28 weeks of gestation in the amniocentesis group (n=135) is again a high percentage (5.9%). Even when corrected by subtracting case numbers 11, 172 and 190 it is still 4.4% and without cases 10 and 63, it can be brought down to a minimum of 2.7%. Preselection seems to have had an aggravating affect (most women declined CVS because of the higher abortion risk reinforced by rather compromised previous histories, such as e.g. recurrent late abortions).

5.2.3 Neither CVS nor Amniocentesis

Another caterory substantially contributing to the 'drop-out' group is comprised women 'who canged their minds altogether', declining any antenatal diagnosis: 36 women in total (=17.1%), 27 of them wishing to continue their pregnancy without either chorionic villus sampling (CVS) or amniocentesis and 9 preferring 1st trimester termination of pregnancy instead[1] (see also section 5.2.1). Reasons given varied from 'risk too small compared with risk of test', to 'want to keep my child anyway', 'don't know' and 'don't want termination of pregnancy if the result is abnormal'.

There were no spontaneous abortions is this group and one woman (42 years, husband 62 years) delivered a Down syndrome child. Long-term follow-up is described in section 5.1.5.1, in conjunction with the CVS and amniocentesis group, giving the impression that this group might generally be doing even worse regarding pregnancy performance and fetal outcome than the other two groups.

[1] Although the total AAP group was n=10, one was after CVS.

6 SUPPLEMENTARY STUDIES

6.1 MATERNAL SERUM ALPHA-FETOPROTEIN BEFORE AND AFTER CHORIONIC VILLUS SAMPLING AND AT 16 WEEKS

Feto-maternal haemorrhage (FMH) can occur as a direct result of second trimester amniocentesis, as indicated by increased concentrations of maternal serum alphafetoprotein (MSAFP) (Lachman et al 1977). Consequently, it is established prophylactic practice to give anti-Rhesus (D) immunoglobulin (anti-D) to Rhesus negative women after amniocentesis.

Little is known, however, about whether chorionic villus sampling (CVS) causes FMH and if so, to which extent. Anti-D prophylaxis after CVS is not generally accepted; some established centres (Hahnemann 1987) do not administer it routinely. The reason usually put forward is that the minimum quantity of fetal blood needed to sensitize the mother, would at the same time result in acute fetal death by exsanguination. As this is not usually the case, it is reasoned that FMH, if it occurs at all, is clinically negligible.

Little is also known about whether 10th week CVS interferes with the reliability of 16th week MSAFP screening for neural tube defects (NTD).

Also, low 16th week MSAFP concentrations have been associated with fetal chromosomal abnormalities, especially the Down syndrome (Merkatz et al 1984, Editorial 1985), while high 16th week MSAFP levels are said to be associated with compromised pregnancy performance and fetal outcome (Christiaens et al 1987).

Although the Kleihauer-Betke test has been advocated for the assessment of FMH after amniocentesis (Simpson et al 1984), MSAFP is considered to be a more sensitive indicator for FMH (Lele et al 1982, Warren et al 1985).

Aim of the study

This study was designed to assess

1. The extent of FMH after CVS and the need for anti-D prophylaxis.
2. The influence of 10th week FMH on 16th week MSAFP screening.
3. The association of 10th week MSAFP levels with subsequent pregnancy problems, such as
   - 'spontaneous' abortion
   - pregnancy-induced hypertension
   - antepartum haemorrhage
   - preterm delivery
   - intrauterine growth retardation

   and particularly the relation of MSAFP with chromosomal and other congenital abnormalities of the fetus.

Materials and methods

We took maternal blood samples from a peripheral vein immediately before CVS and between 5 and 15 minutes after CVS at around the 10th gestational week and measured MSAFP concentrations using the AFP-EIA monoclonal

kit from Abbott ™. We considered an increase in MSAFP concentration of 40% to represent a significant FMH, in line with Lachman et al (1977) and Mariona et al (1986).
We used the same method for second trimester MSAFP analysis [2] in our optional MSAFP routine screening programme. These samples were taken in the 16th or 17th gestational week except in three cases (one in the 18th and two in the 19th week).

Second trimester amniotic fluid AFP (AFAFP) measurements were performed in our own Department[3].

Results

We collected 188 complete bloodsamples (i.e. before and after 1st session CVS) for analysis. Pre-CVS values ranged from 2 to 23 ng/ml [4] (mean 7.1; median 6) and post-CVS levels from 3 to 156 ng/ml (mean 18.6; median 12), see Figure 6.1.

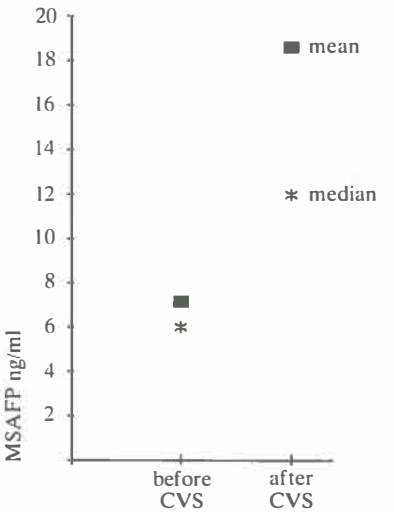


Figure 6.1 Mean and median levels of maternal serum alpha-fetoprotein (MSAFP) before and after 10th week chorionic villus sampling (n=188, SE=0.3 and 1.5, respectively).

[1] Dr.J. Marrink, Department of Internal Medicine, Division of Immunochemistry, University Hospital, Groningen, the Netherlands.
[2] Dr. R. Hagenaars, Government Laboratory for Public Health, Bilthoven, the Netherlands.
[3] Dr. H.W.A. de Bruyn.
[4] All MSAFP values are rounded.

In 115 (61%) the MSAFP concentration rose by 40% or more, see Figure 6.2. Quantitatively, the average rise of MSAFP was 11.6 ng/ml (range 0 - 156 ng/ml) with a corresponding average percentage rise of 213% (range 0 - 3833%). According to Mariona's calculation, this amounts to an average FMH of 0.018 ml (range 0 - 0.24 ml) (Mariona et al 1986).

Second trimester MSAFP values (n=90) were all well below the 97th percentile, except for one patient who aborted the following day (152 ng/ml in the 16th week, see section 5.1.4.2, case no. 7) and another two borderline cases: one 17th week 94 ng/ml (upper limit was 98 ng/ml) who had 8 ng/ml before CVS and 14 ng/ml after (=75% rise), with up to week 20 echoscopically confirmed blood in utero; the other 85 ng/ml, gestational age unreliable, with 17 ng/ml before and 65 ng/ml after CVS, rise 282%. Both pregnancies were uneventful and the fetal outcome was normal.

All 2nd trimester AFAFP levels were normal (n=16, amniocentesis performed after unsuccessful CVS, or as confirmatory AC).

The various subsequent pregnancy problems mentioned above are summarized in Table 6.1 and illustrated in Figure 6.2.

Table 6.1 Pregnancy problems after chorionic villus sampling (CVS) in relation to maternal serum alpha-fetoprotein (MSAFP in ng/ml) before and after CVS, according to pregnancy problem and % Rise (n=188).

MSAFP sample no.	Rise no.	MSAFP before CVS	MSAFP after CVS	Difference	%Rise
'Spontaneous abortion'					
139	21	3	3	0	0%
195	40	6	7	1	17%
148	75	5	7	2	40%
34	100	7	12	5	71%
31	125	10	23	13	130%
133	133	13	33	20	154%
162	175	4	24	30	750%
mean		6.8	17.0		
('normal'		7.0	18.6)		
Pregnancy-induced hypertension					
110	17	4	4	0	0%
88	14	6	6	0	0%
26	34	7	8	1	14%
180	64	3	4	1	33%
12	107	12	22	10	83%
116	113	11	22	11	100%
150	160	8	37	29	362%
173	166	3	19	16	533%
29	167	8	51	43	537%
152	181	7	83	76	1086%
mean		6.9	25.6		
('normal'		7.0	18.6)		

Antepartum haemorrhage[1]

184	27	4	4	0	0%
87	145	8	25	17	212%

(this group not included in Figure 6.2 because of the small numbers)

Preterm delivery[2]

121	44	4	6	1	20%
56	89	6	9	3	50%
22	95	8	13	5	63%

Intrauterine growth retardation

<2.3 percentile:

73	110	17	33	16	94%
45	148	7	23	16	229%
168	152	6	22	16	267%
mean		10	26		

<5 percentile:

23	58	7	9	2	29%
18	61	12	16	4	33%
94	123	5	11	6	120%
mean		8	12		

Congenital abnormalities

Trisomy 21:

60	60	6	8	2	33%
66	16	32	11	9	450%
198	169	2	13	11	550%
112	180	5	59	54	1080%
153	187	4	156	152	3800%
mean		3.8	49.4	41.8	1182%
('normal'		7.0	18.6)		

69,XXX:

20	31	9	10	1	11%

XO:

11	37	6	7	1	17%

Fallot's tetralogy:[3]

74	87	6	9	3	50%

Cheilognathopalatoschizis:[4]

37	68	11	15	4	36%

1 Excluded sample no. 84 and no. 86 (slight spotting from cervix and vulva respectively) and no. 168 (only little APH, classified as IUGR).
2 Sample no. 74 excluded: Fallot, classified under congenital abnormalities.
3 Not classified under preterm delivery.
4 Not classified under small-for-date.

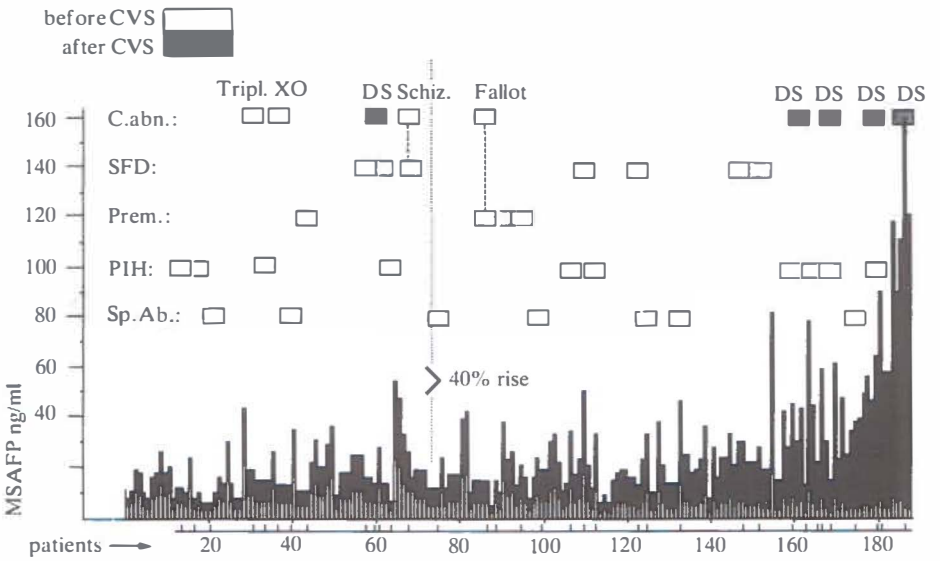


Figure 6.2 Maternal serum alpha-fetoprotein (MSAFP) before and after 10th week chorionic villus sampling (CVS) according to percentage rise. Each bar represents one case (n=188), the white part of the bar the level before CVS and the black part the concentration after CVS in ng/ml. Each square block represents a patient with the condition described (the two dotted lines connect the same patient).
(note: C.abn.=congenital abormalities; SFD=small-for-date; Prem=preterm delivery; PIH=pregnancy-induced hypertension; Sp.Ab=spontaneous abortion; Tripl=triploidy; X0=Turner's syndrome; DS=Down's syndrome; Schiz=cheilognathopalatoschizis; Fallot=Fallot's tetralogy).

Discussion

A rise in post-CVS MSAFP of 40% or more was seen in 61% of our patients. The average FMH of our total group of 188 women was calculated as ranging from 0 to 0.24 ml (average 0.018 ml). Generally our figures compare well with Mariona's small group of 21 women (Mariona et al 1986):

	Mariona n=21	Mantingh n=188
MSAFP in ng/ml before CVS, mean	4.11	7.1
S.E.M.	0.76	0.3
after CVS, mean	17.65	18.6
S.E.M.	4.66	1.5
average rise quantitatively in ng/ml	16.6	11.6
corresponding with	0.025 ml	0.018 ml
(pre-CVS range	0.00- 15.00	2- 23)
(post-CVS range	1.65- 76.20	3- 156)
(%change range	-35 -2300%	-40-3833%)

While Mariona's maximum FMH did not exceed 0.1 ml, our maximum level was over twice as high (0.24 ml). So it is not a question of whether FMH occurs after CVS, but whether the extent of FMH warrants post-CVS anti-D prophylaxis on a routine basis.

The chance of Rhesus isoimmunization occurring with these small amounts is said to be small. Mollison (1986) has stated that Rhesus isoimmunization can occur with fetomaternal bleeding of 0.25 ml or more but adds that some patients may become sensitized with less. Zipursky and Israels (1967) were more specific: 3% chance with a FMH of 0.1 ml (Zipursky and Israels 1967). One could therefore easily conclude that with our average FMH of 0.018 ml, the risk is approximately 5 times lower, i.e. the risk of sensitization is probably negligible. On the other hand, if supposedly a minimum of 0.1 ml is needed, corresponding with 66.4 ng/ml (see Mariona's calculation above), then 6 patients (3.2%) in our series were at risk for sensitization. Or, in other words, a Rhesus negative woman undergoing CVS has a 3% chance of FMH with $\geq$0.1 ml. A FMH of this size in itself carries a 3% risk, which means that 9 out of 10,000 Rhesus negative women will become sensitized, say 1 in 1000. In our series 1 in 6 women where Rhesus negative. Therefore, with our present 300 CVS annually, it will take some 20 years of sustained anti-D prophylaxis to avoid one isoimmunization. This at a cost of appr. 50,000 guilders (if one restricts prophylaxis to the injection of one 75 µg vial only) and with the risk of potential serious side-affects as described in section 5.1.4.6.

Although Marioni et al (1986) have suggested that 50 µg anti-D is sufficient to avoid sensitization, they nevertheless recommed 300 µg anti-D every three months prophylactically throughout pregnancy, reasoning that CVS could trigger persistent chronic leakage of fetal blood causing sensitization in the long-run. This seems rather unlikely as we found normal 16th week MSAFP concentrations in all unaffected pregnancies. Also, our current practice of giving anti-D in a single dosage of 200 ug overshoots direct FMH after CVS almost a 100 times (10 µg anti-D neutralizes 1 ml fetal blood, Bennebroek Gravenhorst 1984).

Another reason sometimes expressed for not giving anti-D (besides 'the-risk-is-too-small' one), is that the quantity of fetal blood needed for sensitization is, so early in pregnancy, relatively so large that the fetus would immediately bleed to death. This not being the case, such a FMH can't have happened either.

We doubt whether this reasoning holds true. Although the blood volume in a 10th week 5G fetus might still be minimal, the placenta at that gestational age is plm. 20G and contains approximately 2 ml fetal blood (Boyd 1987). Taking 0.1 ml FMH as the minimum amount of fetal blood needed to possibly cause immunization would mean a loss of not more than 5% of the total fetal blood volume minimally present.

Procedure-related factors associated with FMH have been adequately investigated before (e.g. no correlation between a significant rise in MSAFP and gestational age, weight of specimen or blood staining of biopsy material, but only a significant correlation with the number of attempts at obtaining a biopsy, Warren et al 1985).

As for the association between a rise in 10th week MSAFP and subsequent

pregnancy problems, all are rather evenly distributed, as shown in Figure 6.2 [1]. A shift is only seen in the small-for-date category: the smaller the fetus, the greater the shift to the right (all three <2.3 p were in the ≥40% rise category).

In our investigation of the relation between MSAFP concentration and congenital abnormalities, 5 Women with fetal Down syndrome (all trisomy 21) were found to have MSAFP concentrations of 2, 2, 4, 5 and 6 ng/ml, respectively. Figure 6.3 shows the distribution of these levels in comparison with 183 women without fetal Down syndrome (DS).

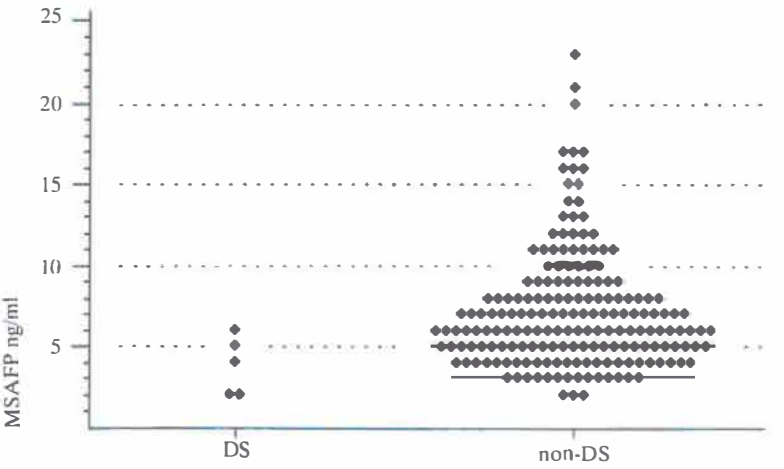


Figure 6.3 Distribution of first trimester maternal serum alpha-fetoprotein levels (MSAFP) in women with fetal Down syndrome (DS) and without fetal Down syndrome (non-DS).

At a 2 ng/ml MSAFP level the increased incidence of DS is no longer accidental (Fisher's exact probability test, P=0.005). Of course, more data are required for the assessment of the specific odds for DS at different MSAFP concentrations (the present 1:2.5 at ≤2 ng/ml might well be too high).

We did not employ a modified MSAFP testing method better suited to the low-range values as some investigators have suggested (Mariona et al 1986, Milunsky 1987), as we considered the accuracy of our technique of ±10% over the whole range was adequate to serve our initial purpose of investigating post-CVS FMH. But also in retrospect we feel confident in assuming that low 10th week MSAFP values considerably increase the likelihood of trisomy 21 (even at ≤6 ng/ml: 5 women with fetal Down syndrome and 96 without fetal Down syndrome gives a 5.2% risk in a group with an average risk for chromosomal abnormalities of around 1% when only factors such as e.g. maternal age are taken into account).

[1] Antepartum haemorrhage omitted: 2 real cases only.

The use of MSAFP as a screening factor for Down's syndrome (DS) later in pregnancy has been advocated before. Not only to select young women with a risk comparable with advanced maternal age (Cuckle et al 1984), but also to identify older women who run a lower risk than that based on their age alone. Tabor et al (1987) suggested combining MSAFP levels and maternal age in iso-risk curves applicable to all pregnant women, irrespective of their age. They calculated that the amniocentesis (AC) detection efficiency for Down's syndrome increases from 28% when based on maternal age alone (35 years and over) to 53% when based on a $\geq$1:400 iso-risk curve for all pregnant women in Denmark. This looks attractive because the specificity only drops from 0.93 to 0.91 and the extra number of AC to be performed is still manageable (from 6.9% to 9.4% of all pregnant women).

However, the disadvantages of conducting such a screening test relatively late in pregnancy are evident. A risk discovered during pregnancy is harder to cope with than a risk known beforehand (Farrant 1980) and the subsequent long wait for the AC result increases parental anxiety. A screening method based on first trimester MSAFP values could greatly reduce this problem. It could be considered more or less in line with other screenings done at the first antenatal visit and, more important, it can be followed by a confirmative CVS giving a result in a matter of days.

Little is known about 10th week MSAFP values in this respect. Brambati et al (1986) were the first to report 17 fetal aneuploidies in association with first trimester MSAFP: all trisomies 21 had MSAFP concentrations below the median (the normal MSAFP range 8-12 weeks constructed from values in 446 normal pregnancies). In the remaining 9 other aneuploidies, 2 were above the median. Odds ratios were not given. Barkai et al (1987) confirmed this by adding 3 trisomies 21 with low MSAFP (expressed in M.O.M, multiples of the median): 0.5, 0.6 and 0.7, respectively. At the Chicago CVS Conference 1987, Milunsky presented data from a joint American-Italian excercise comprising a group of 540 women in whom a total of
22 chromosomal disorders occurred. With a median of 6 ng/ml MSAFP he found:

	$\leq$0.5 M.O.M.	$\leq$0.6 M.O.M.
Total	32	59
Number of trisomies	3	7
Number of all chromosomal disorders	3	8
Odds trisomies	1:11	1:8
Odds all chromosomal disorders	1:11	1:7
(odds Down's syndrome	1:16	1:20)

He calculated that in his situation a cut-off point of $\leq$3 ng/ml would give a 30% detection efficiency viz. 10% with 16-18th week amniocentesis based on advanced maternal age alone.

MSAFP 2 ng/ml occurred 5 times in our series of 188 women (2.7%). Taking

this ≤2 ng/ml as a cut-off point for screening would result in an increase of 5000 CVS or amniocentesis (AC) procedures annually in the Netherlands at most (in the unlikely event that all 190,000 pregnant women would want to be screened and would want confirmation by CVS or AC as well). Even with a cut-off point this low, the percentage of our pregnant population selected in this manner would already rise from approximately 3 to 5.5%. Although this may seem small in comparison with the numbers involved in other antenatal screening programmes, we would still be unable to deal with such a rise at such short notice as it would mean almost doubling our annual figure of 5744 total CVS/AC for 1987. The demand for such a programme, however, will probably be lower and more in line with the 30% response for antenatal diagnosis for advanced maternal age now existing in the Netherlands (Kloosterman 1988), reducing the numbers to manageable proportions.

Our first figures indicate that first trimester MSAFP has a good screening potential for DS, possibly as good as 16th week MSAFP in terms of detection efficiency for DS and probably superior in terms of acceptability. A cut-off point ≤2 ng/ml MSAFP seems to be a realistic starting point, although the figures are still too small to allow MSAFP-specific odds to be calculated.

In conclusion:

1. Feto-maternal haemorrhage after CVS can be considerable (up to 10% of the estimated fetal blood volume).
2. A single dose of 50 µg anti-D should be more than adequate. It would cost approximately 50,000 guilders to avoid one sensitization (±6000 CVS).
3. First trimester CVS does not interfere with 16th week MSAFP screening for NTD.
4. It seems unlikely that antepartum haemorrhage, pregnancy induced hypertension, preterm delivery and spontaneous abortion are associated with the level of post-CVS MSAFP. For small-for-date infants this is less clear. For trisomy 21 the MSAFP rise distribution pattern was significantly different.
5. The distribution of 10th week MSAFP levels in pregnancies associated with fetal Down syndrome differed significantly from non-DS ones.

6.2 THE IMMEDIATE EFFECTS OF CHORIONIC VILLUS SAMPLING ON FETAL MOVEMENTS

(adapted from Boogert A, Mantingh A, Visser GHA, 1987, see references Chapter 9.

Chorionic villus sampling has become an increasingly popular technique for early prenatal diagnosis. More than 10.000 investigations have been performed worldwide to date (Jackson 1986/15)[1]. So far the fetal consequences of this procedure have been assessed in terms of the abortion rate, fetal growth as observed via serial ultrasound, and perinatal outcome (Gustavii 1984; Brambati et al 1985; Hogge et al 1985). With real-time ultrasound equipment, distinct patterns of fetal behavior have been described from 7 weeks of gestation onward (de Vries et al 1982). We attempted to determine the acute effects of this invasive diagnostic procedure on fetal motor behavior by means of two 30-minute observations taken immediately before and after chorionic villus sampling.

Patients and methods

Ten multiparous women were selected at random from those referred for chorionic villus sampling. After receiving a detailed explanation of the investigation, they agreed to take part in the study.

Continuous real-time ultrasound images were obtained and recorded on videotape for 30 minutes immediately before and 30 minutes directly after chorionic villus sampling with an Aloka SSD-256, which was equipped with a 3,5 MHz linear array transducer. The fetuses were usually visualized in the mid- or parasagittal longitudinal section. The analysis of fetal motility was carried out during playback of the video recordings in the presence of an independent observer to minimize bias and reduce interobserver error. The incidence and duration of the different movements were marked on an event recorder (model 7754a, Hewlett-Packard, Palo Alto, Calif.) by handheld push buttons. General movements, startles, hiccups and breathing movements were analyzed according to the criteria described by the Vries et al (1982). General movements were expressed as a percentage of the time they were present; the other movements were simply counted. In case the recording time differed slightly from 30 minutes, the incidence of movements was normalized by conversion to 30 minutes. The results were analyzed by the Wilcoxon signed rank test.

[1] This research was carried out during the first year of our CVS service.

Figure 6.4 Compressed actogram of general fetal movements before and after chorionic villus sampling (CVS) in the 10 cases.

Table 6.2 Clinical data on the 10 women, as well as the incidence (percentage of time or number per 30 minutes) of fetal movements before and after chorionic villus sampling

Case No.	Age (yr)	Duration of pregnancy (wk and days)	General movements (% of time)		Startles (n)		Breathing movements (n)		Hiccups (n)	
			Before	After	Before	After	Before	After	Before	After
1	37	10w	25	18	8	18	24	39	41	51
2	36	11w+1d	24	27	1	1	0	2	0	1
3	38	9w+1d	28	25	5	10	0	0	0	2
4	41	9w+6d	15	20	11	4	13	2	35	11
5	26	10w+1d	20	17	4	9	4	0	4	11
6	38	9w+5d	23	24	8	8	1	0	0	1
7	38	9w+6d	21	24	9	7	2	0	10	10
8	39	9w+5d	20	22	5	3	0	0	11	21
9	36	9w+5d	20	19	19	8	10	0	24	33
10	40	11w+5d	27	19	7	11	22	11	1	9
Mean	37	10w+1d	22.3	21.4	7.7	7.9	7.6	5.4	12.6	15.0

Chorionic villus sampling was performed with a trophoblast aspiration catheter (Trophocan), which was introduced transcervically under ultrasound guidance. A maximum of three aspirations per session were carried out to obtain approximately 20 mg net weight of good quality villi for analysis. No anesthetic agents or maternal sedation was used. The perineum, vagina and cervix were thoroughly cleansed with povidine iodine solution before the procedure was begun.

Results

The study population comprised part of the first 100 diagnostic chorionic villus samplings carried out in our department. The overall abortion rate in this series was 3%, with successful sampling in 96 patients. Interestingly, two of the three patients who aborted spontaneously after chorionic villus sampling were part of the study population (cases 1 and 10, Table 6.2). In the latter case the gestational sac was accidentally ruptured during the procedure. The results of the procedure were otherwise unremarkable. A mean weight of 36 mg of chorionic villi was obtained after an average of 1.7 aspirations between 9 and 12 weeks of gestation. No abnormal karyotypes were found in the series.

The statistical analyses of fetal movements before and after chorionic villus sampling did not show significant differences for all four modalities analyzed (Table 6.2). In the two cases ending in a spontaneous abortion (7 and 14 days after chorionic villus sampling), no significant change was noted immediately after the procedure, although there was a tendency toward reduced general movements in these fetuses.

The actogram of general fetal movements is displayed in Figure 6.4, which shows a high overall incidence of movements both before and after chorionic villus sampling. It also shows that the incidence of general movements directly after chorionic villus sampling was the same as at the end of the second 30-minute recording.

Discussion

The only other study on the immediate effects of chorionic villus sampling on the fetus to date was conducted by Löfberg et al (1985), who describe transient fetal bradycardia during direct-vision sampling by means of the extra-amniotic instillation of 150 ml of physiologic saline. This effect appears to be the result of a rise in intrauterine pressure and consequently is not relevant to our study.

Fetal movements can be observed from 7 weeks postmenstrual age (de Vries et al 1982). There is an early emergence of specific movement patterns; at 16 weeks 15 distinct patterns are already present, which closely resemble those observed in preterm and term newborn infants (de Vries et al 1982). When the different movement patterns are rank ordered according to their first appearance, a specific sequence emerges. It has been demonstrated that in embryos and fetuses of type-1-diabetic women, there is a delay of about 1 week in the first occurrence of these movements, except for breathing (Visser et al 1985). Furthermore, it appears that in anencephalic fetuses, movements

patterns are (already) qualitatively abnormal in the first half of pregnancy (Visser et al 1985).

In Figure 6.2 the compressed actogram of general movements shows that there is already a high base rate of spontaneous fetal movements at 9 to 11 weeks. With respect to these and the other movements studied, the data show that chorionic villus sampling does not stimulate movements or alter the intrauterine situation in such a way as to decrease fetal movements. The latter is known to occur in threatened abortions (Schmidt et al 1981). In the two cases of a subsequent spontaneous abortion, the incidence of general movements but not the other movements studied was slightly decreased after chorionic villus sampling. However, more data are required to determine the significance of this finding.

6.3 CHORIONIC VILLUS SAMPLING (CVS) CHARACTERISTICS, PLACENTA HISTOPATHOLOGY AND PREGNANCY OUTCOME

The realization that, for the first time ever, chorionic villus sampling offers the opportunity to link a tenth week biopsy of fetal material with the same continuing pregnancy and subsequent outcome, is very intriguing. Before we had to work with 'normal' material from pregnancies terminated for psychosocial reasons, or with 'abnormal' material from spontaneous abortions. Could particular morphological abnormalities, or deviations from the normal developmental patterns of trophoblastic villi, reflect particular pregnancy problems or fetal abnormalities? Although very tempting indeed, we soon realized that such a line of research would be too ambitious within the scope of a thesis dealing with other aspects of CVS as well. We also felt that some other questions had to be addressed first, for example with regard to one of the patients' main worries: 'How is my baby going to grow properly if you remove pieces of the placenta so early in pregnancy?'

We referred to Blakemore (1985) above in section 2.1. She specifically mentioned intrauterine growth retardation, premature rupture of membranes, abruptio placentae, placenta accreta/increta, placenta praevia, and pregnancy-induced hypertension among the main issues to be addressed in future as potentially CVS-induced problems 'because part of the placenta is removed at sampling'. We therefore decided to concentrate on the issues first at hand while at the same time building up a chorionic villus bank for future research when numbers would have accumulated to the extent of being able to compare sizeable subgroups of patients with particular pregnancy problems to specific morphological and other features of chorionic villi themselves, so as to make the effort really worthwile.

Thus taking first things first and not wanting to spend more than a year chasing post-CVS placentae, we considered approximately 50 to be enough for some preliminary conclusions. Despite many women delivering at home at quite some distance from our centre, we were able to collect this number in the allotted time, thanks to the cooperation of those who assisted at the delivery. Some husbands were even so determined that they came over themselves to deliver 'their' placenta by hand.

In conclusion, this study tries to answer the question of whether or not it is likely that CVS causes specific placental abnormalities from which some possibly related problems, such as intrauterine growth retardation, might be explained.

Materials and methods

During the period of approximately one year, July 1985 - August 1986 following our first CVS, 48 placentae were sent to our department of Pathology[1] for further investigation by Dr. C.J.F. Schoots. In order to examine the placentae

[1] Head Prof.Dr. J.D. Elema. Department of Pathology, University Hospital, Groningen, the Netherlands.

as thoroughly as possible for any mark left by the preceding CVS, specific information was also provided i.e.:
- size of sample in mg as estimated at CVS,
- quality of sample, i.e. whether the specimen consisted of good quality trophoblastic villi only, or whether either poor/peripheral villi, blood, decidua, or chorionic membrane were aspirated as well,
- placental site of sampling (central/near cord insertion, paracentric or at the placental edge,
- total number of CVS sessions and cannula insertions per session,
- uterine abnormalities such as e.g. fibroids, discrepancies ultrasound viz. gestational age, etc.

Sample left-overs not needed by the geneticist for karyotyping were placed in our villus bank for future reference.

Detailed information was given about any pregnancy problem. Apart from specific fetal abnormalities, we obtained the following standard information about the respective babies: gestational age, weight, height, head circumference, biparietal diameter, chest circumference, abdominal circumference and length of feet.

Results

The weight of these 48 placentae ranged from 260 G to 875 G, with an average of 530 G. Fourteen were classified by our pathologist as 'normal'. An overview of all abnormalities observed is given in Table 6.3.

Table 6.3 Placenta histopathology in post-CVS placentae (n=48) (note: some placentae had more than one abnormality; percentages relate to placental volume)

– Thrombi (from 1 to 10 in total, all	<5%)	15
– Infarctions	<5%: 4 >5%<10%: 3	7
– 'Ischaemic changes'		6
– Immature villi	I: 3 II: 2	5
– Chorioamnionitis		3
– Haemorrhagic endovasculitis (HEV)		3
– Septal cyst		2
– Chorioangioma		1
– Feto-arterial thrombosis		1
– Blood-filled cavity		1
– Intervillous haematoma		1
– Villitis of unknown aetiology (VUA)		1
– no abnormalities		14

Corrected for gestational age and parity, 6 placentae were under the 10th percentile for weight. Four corresponding women also delivered a small-for-date infant (again <10th weight percentile), while the two woman with a small-for-date placenta produced 2920G and 3240G infants. In other words:
– 4 below 10p placentae resulted in small-for-date infants
– 2 below 10p placenta resulted in an appropriate-for-date infant
– 4 below 10p infants had over 10p placentae

Table 6.4 Small post-CVS placentae (<10p) and/or small-for-date infants (<10p) in association with CVS-related parameters, e.g. weight of villi, number of insertions, etc., as well as details of pregnancy follow-up.
(HEV=haemorrhagic endovasculitis, PIH = pregnancy-induced hypertension)

Record number	Child percentile	CVS tions	No. of Inser-/after	aFP before	Pregnancy problems	Placenta
no. 98	<2.3p	15 mg villi only	2	7/23	none	260 G, macro-infarctions <10%, ischaemic changes
no. 188	<5p	48 mg villi, a little blood and decidua	2	5/11	none	300 G, macro-infarctions <10%, ischaemic changes
no. 82	<5p	25 mg villi, minimal blood	1	11/15	none	330 G, normal
no. 46	<5p	55 mg villi only	1	12/16	?cervical incompe-tence: cerclage	445 G, thrombi <5%
no. 69	<10p	44 mg villi only	1	8/51	PIH+ catapresan	470 G, slight ischaemic changes
no. 31	<10p	60 mg villi, small piece of chorionic membrane	3	12/22	PIH	485 G, septal cyst
no. 112	<10p	50 mg villi, a little blood	1	–/–	none	490 G, septal cyst
no. 34	<10p	52 mg, small piece decidua	2	6/13	none	395 G, normal
no. 38	>10p	0 + 23 mg in 2 sessions	3+2	–/–	none	365 G, one ischaemic spot
no. 110	>10p	28 mg villi only	1	14/10	none	330G, 1 thrombus + HEV

Discussion

Three of the four women who delivered a <5p small-for-date infant also had a very small placenta. The other woman's placenta was of normal size. All four had undergone a completely uneventful CVS with small to normal amounts of total villi weight obtained and no or insignificant other tissue fragments aspirated in not more than 2 insertions without complications directly afterwards, or later in pregnancy. (The only woman in this series with a difficult CVS, patient no. 38, had a 2920G child despite a 365G placenta!). These findings are not consistent with the idea of CVS causing intrauterine growth retardation. Of course, the placenta-fetus weight ratio at around the 10th week gestational age, 4:1[1] already suggests one could easily do without the average 25 - 50 mg of villi taken at sampling. Other factors such as difficult or traumatic CVS procedures, multiple sessions, aspiration of decidua or fragments of chorionic membrane, could, however, interfere with a pregnancy in a negative way. Yet follow-up results discussed in previous sections do not lend support to this. Despite many abnormalities seen at histopathological examination, they were not different from the pattern found in a general population. Scars or other marks that might have indicated a healed post-CVS leasion, could not be traced in these placentae. Remarkably, the most difficult (and failed) CVS in this placenta group was followed by a full term 4945 G / 56 cm boy being born, after three CVS sessions with 74 mg poor villous material obtained in 8 aspirations in total, followed by a difficult amniocentesis, but no problems whatsoever thereafter.

In conclusion, no post-CVS placental abnormalities were found that could possibly be interpreted as CVS-related lesions. Therefore, placenta-related problems, such as intrauterine growth retardation, are unlikely to be a direct result of CVS. Intriguing research into the possible relationship between 10th week villous tissue characteristics and pregnancy performance lies ahead.

[1] Approximately 5G fetus and 20G placenta.

6.4 CHORIONIC VILLUS SAMPLING OR AMNIOCENTESIS?

Aspects of decision making by women and their experience. (adapted from Keppels M, van der Velde AS, Tijmstra Tj, Mantingh A, 1987, see references Chapter 9)

From the introduction of chorionic villus sampling (CVS) at our hospital in November 1984, pregnant women wanting antenatal diagnosis had a choice between CVS and amniocentesis, provided they came early enough (10 completed weeks of gestation at the latest). Before November 1984 amniocentesis was the only option. During intake, information on CVS and amniocentesis was given verbally as well as in writing (see section 3.1 and Appendix 11.4).

Twenty women, 10 from the CVS group and 10 from the amniocentesis group were interviewed in order to gain a better insight into the ways of decision making by the women and their experience with both types of antenatal diagnosis. After all, apart from the pros and cons inherent to the tests themselves, the mere introduction of CVS at first sight seemed to have complicated matters considerably for prospective parents as first: they now had to make two decisions, 1. antenatal diagnosis or not and 2. if yes, CVS or amniocentesis and secondly: all this at rather short notice (less than half the time available compared with when amniocentesis was the only option and in practical terms usually just a week or two after a positive pregnancy test).

This study was a joint venture with the Department of Medical Sociology (Dr. Tj. Tijmstra with co-workers M. Keppels and A.S. van der Velde) (Keppels et al 1987). The women were interviewed at their homes, the discussions being tape recorded.

Why do you prefer CVS?

Nine out of the ten women in the CVS group had been pregnant before. Two of them had undergone amniocentesis in a previous pregnancy. Indications for CVS were maternal age and previous history, each five times. Although they all had an increased risk of delivering a chromosomally abnormal child, most of them did not really know how high the risk was (*'Actually I have not given much thought to how much at all'. 'Just the fact there is a risk'*).

All the women had already talked over the possibility of CVS with their general practitioner (GP) or gynaecologist, often having taken the initiative for such a discussion themselves. Some doctors were still somewhat unfamiliar with CVS (*'He didn't know anything about that test, well, actually he knew it existed but thought it was one of those women's magazine stories'*).

From the beginning these women were rather enthousiastic about CVS. One of them even said that without CVS she surely would not have dared to become pregnant again (*'We would definitely have stopped'*). Some pointed out that

they felt that they 'ought to make use of it' (*'If you have such a child after all, you realise then that you could have been tested...., well, it encourages you to make full use of the opportunity'*).

The group was very satisfied with the intake procedure at the hospital. They said to that they had been well informed about the pros and cons of both CVS and amniocentesis. The choice in favour of CVS was usually made quickly, although some had hesitated slightly because it was 'something new' (*'I would rather have been number 100 than number 5'*).

For all ten women the timing of the test so early in pregnancy was decisive in preferring CVS (*'That's the best thing about CVS: they can do it very early on so it will be far easier to give the pregnancy up if necessary'*). Many women also stressed the advantage of 'privacy' (*'At such an early stage you can just keep it a secret, keep mum about it for your family: you won't get comments about it regarding religion or whatever if you would have it terminated after all'*). Another pro mentioned by many was the short wait for the results.

Disadvantages of the test were hardly mentioned. Only one woman explicitely pointed out the abortion risk (*'I read it was something like 3 per cent. Well, I think that's probably responsible for part of the miscarriages already known'*). All the women were of the opinion that the CVS procedure itself could have been far worse (*'It's nothing really'*). The relaxed atmosphere was often mentioned (*'It was a very friendly atmosphere, they really made you feel like part of the team'*). The actual period of waiting for the results was experienced as difficult. One woman had clearly suffered mentally and physically (*'They also completely open up the womb don't they? When I came home I went to bed and my womb contracted. I think it was probably all nerves'*).

All the women knew beforehand on which day they would receive a telephone call about the result. That was a nerve racking moment (*'I had hot and clammy hands when he phoned'*). Some said they would have preferred to have received the result by mail. Hearing the result (normal for all women) gave them a great deal of relief. From then on they could really be happy about their pregnancy. Eight women said they experienced their pregnancies more positively since the test (*'I did what I wanted to do, I got a lot quieter, it's a nice idea to know that I don't have thát risk anymore, at least'*). Two women said that they did not feel much difference between the period before and after CVS.

One of them had a serum alpha-fetoprotein screening at 16 weeks of pregnancy (result normal). Three said not to have known about the possibility. The remaining six had declined the test (*'If you start searching you can keep on searching'*).

Why do you prefer amniocentesis?

In this group of ten women, nine had been pregnant before and one of them had undergone amniocentesis in a previous pregnancy. In eight of them the

indication was advanced maternal age. Nevertheless, they were unable to say what precise risk they actually ran for congenital abnormalities (*'You just know you run a risk, so, as such, its extent does not matter very much'*). All the women were aware of the possibilities of antenatal diagnosis before their pregnancy. Thus, most of them had decided early on in pregnancy that they would like to undergo such an examination. One woman, however, did not take it so much for granted (*'My womb was rather restless so it was better not to have the test done. Later they told me that it would be possible after all. I found it very annoying that I had to face that choice again'*).

Most of the women knew about CVS too and often mentioned it themselves to their GP or gynaecologist, who were of limited help as regards further information (*'He didn't know that much about it. He said they'll tell you about it in Groningen'*) One woman had heard about CVS for the first time during intake at the hospital (*'First you doubt whether you want to have it examined anyway; then they suddenly ask you to decide between one of two methods!'*). For most of the women the choice between amniocentesis and CVS was not a difficult one. Before intake they had already more or less decided in favour of amniocentesis. (*'It was already clear in my mind that I wanted amniocentesis'*). Information during intake at the hospital did not change this preference. As far as CVS was concerned these women made remarks like: *'It was actually a bit of an uncertain sort of test'*, *'It's still in its infancy'*, *'You read those fantastic stories in magazines about it but you really have to tone them down'*.

The abortion risk was a very important and for many decisive argument against CVS. All the women concerned experienced this factor as very threatening (*'I want so much for this pregnancy to be allright, to do all the right things, carry it out properly. Then I considered CVS to have a greater abortion risk after all'*). Some also mentioned the 'limited nature of the test': it does not give information about neural tube defects. If you want to find out this information, a supplementary test has to be done later (*'So, for a couple of weeks, you are still going to have that uneasy feeling of I'm pregnant but not yet actually for real'*). The possibility of the test being unsuccessful was also mentioned as a drawback fairly frequently (*'In that information stencil they wrote that if something went wrong when they were sucking up the tissue you had to have an amniocentesis at 16 weeks after all'*). Some women mentioned the possibility of growth retardation of the fetus (*'That test could possibly mean that the baby grows too slowly: that gives me an uncertain feeling, because imagine if it turns out that it hasn't grown properly, you will always be thinking that it's your own fault'*). For these women these arguments outweighed the advantages of CVS (which were surely recognised as well).

Some of the women described the period until the amniocentesis as being rather difficult (*'I couldn't be truly pregnant, I found it terribly difficult'*). Although many had dreaded the investigation, they were pleasantly surprised that it was not all that bad. (*'It all looks a bit gruesome but actually it doesn't amount to much'*).

Waiting for the results was rather stressing. One woman in particular had experienced a very hard time (*'I have never cried so much in my life than during that period, every night I cried myself to sleep'*). Around this time the baby's movements are felt for the first time, making the women realise the consequences of a possible termination of pregnancy (*'Thinking that if it's not allright I will have to have it removed. I have to because that's what we'd both decided. But I do not think that I would ever have got over it'*).

Hearing the result was a great relief. Thereafter, most of them found their pregnancy more enjoyable, especially those 'who had hardly dared to be happy before'. Some were of the opinion that things had not changed much (*'You never know how it will turn out for sure, of course, something else might go wrong as well'*). In a possible future pregnancy, three women said that they would prefer not to undergo amniocentesis again, but would rather go for CVS. Four older women were inclined to agree, and would definitely choose CVS if the abortion risk could be more precisely defined by then (and was 'acceptably' low).

Discussion

We have described the experience of pregnant women who had to decide between two types of antenatal diagnosis. Both the existing method, amniocentesis and the newly developed chorionic villus sampling, CVS, offer a number of advantages as well as disadvantages. Those who preferred CVS did so mainly because this test provides information early in pregnancy: a possible termination of pregnancy was condidered to be less taxing at such a time, with the added possibility of keeping it secret from others.

We had the impression that the CVS group of women took a somewhat more realistic and 'business-like' view of pregnancy than the amniocentesis group. The latter set a high value of importance on their pregnancy and considered the CVS-related, imprecise, but probably increased abortion risk to be very threatening. They accentuated – partly because of this? – some other disadvantages as well.

The amniocentesis group clearly had no idea about the feelings that can arise when a woman is confronted with the possibility of having to have her pregnancy terminated late in pregnancy. Admittedly, this is not an easy thing to anticipate for someone who has only been pregnant for a couple of weeks. This also probably explains why many women in the amniocentesis group would prefer to undergo chorionic villus sampling in a possible future pregnancy. This point should receive particular attention during the counselling of patients on these two diagnostic methods.

7 CONCLUSIONS AND RECOMMENDATIONS

1. The overall utilization of antenatal diagnosis in the Netherlands by women with an increased risk of fetal chromosomal disorders (maternal age ≥36 years) has not changed appreciably over the last 5 years. It has remained stable at ±30%, representing a low detection efficiency for fetal chromosomal disorders in the general population of ±10%. The respective percentages in the provinces in the north of the Netherlands, Groningen, Friesland and Drenthe, however, have deviated: 34%, 24% and 33%, respectively, in 1986 compared with 23%, 19% and 23% in 1978.

2. In order to 'improve' this 10% detection efficiency, one could either (a) try to improve the antenatal diagnosis utilization rate of women ≥36 years of age, (b) lower the ≥36 year maternal age limit, or (c) try to identify young women who run a risk which is comparable with advanced maternal age. Option (a) is not very promising (see conclusion 1), option (b) could be tried but is not attractive (see below), leaving option (c) as the one that should be pursued (see conclusion 3).

The maternal age limit restriction of ≥36 years is based on the assumption that at this age the risk of fetal chromosomal abnormalities becomes larger than the average population risk of these anomalies (0.5%). Incidentally, this risk also becomes larger than the amniocentesis-related abortion risk (usually put at 0.5%). This procedure-related risk is derived by subtracting the natural background abortion risk from the total number of abortions occurring after antenatal diagnosis. We found that our chorionic villus sampling (CVS)-related abortion risk was ±5 times higher. However, the total number of abortions after CVS remained relatively low (5.0%). We think that this is because the natural background abortion risk is lower than assumed, rather than the procedures being so safe.

3. The most attractive policy to 'improve' the current ±10% detection efficiency is option (c) mentioned above: trying to identify young women who run a risk which is comparable with advanced maternal age.

We confirmed that the use of first trimester maternal serum alpha-fetoprotein (MSAFP) as a marker for fetal Down syndrome is promising and should be pursued on a larger scale to enable the calculation of specific odds at different MSAFP concentrations. We suggest taking ≤2 ng/ml MSAFP as an initial cut-off level, the more so, as reliable fetal karyotyping by chromosome analysis of fetal lymphocytes from maternal blood is not to be expected in the near future.

4. Although the introduction of CVS might have increased the attractiveness of antenatal diagnosis, it did not result in an increased demand by women

eligible because of advanced maternal age. The steep rise in the number of women utilizing antenatal diagnosis was caused by the maternal age limit being lowered from 38 years to 36 years, in conjunction with a strong increase in fertility in this ⩾36-year-old maternal age category, the actual response rate remaining more or less the same.

It did, however, cause a shift in the overall antenatal diagnosis referral ratio by the 1st and 2nd health care echelon from ±30-70 to ±40-60 (and for CVS alone even to 50-50).

5. The incidence of false-positive CVS results casts doubt on the status of CVS as a diagnostic procedure and our findings confirm discordant results reported by others. Strictly speaking, this implies that all abnormal CVS results should be confirmed by follow-up amniocentesis and this in turn would mean that CVS should be regarded as a screening procedure only, i.e. requiring further tests to verify its results.

The occurrence of false-negative results, though very rare, strengthens this view.

6. Feto-maternal haemorrhage (FMH) caused by CVS (as indicated by altered maternal serum alpha-fetoprotein (MSAFP) levels in maternal blood after CVS), can be considerable, but do not seem to increase the likelihood of complications later in pregnancy, or to interfere with 16th week MSAFP screening for neural tube defects.

Nevertheless, it confirms the need for post-CVS anti-D prophylaxis in Rhesus negative women, however small the chances of isoimmunization caused by FMH may be and despite the potentially serious side-effects of anti-D.

It is our impression that the association of large FMH with fetal Down syndrome (DS) probably marks DS-pregnancies that would have ended in a spontaneous abortion anyway.

7. Before and during CVS microbiological and histopathological characteristics were not associated with any particular pregnancy problems or specific placental pathology.

Fetal motility patterns, used as a parameter for fetal well-being, were normal before and after CVS, even when the sampling procedure had evidently been quite traumatic.

8. Since the introduction of CVS, couples usually have to make a decision about antenatal diagnosis soon after the pregnancy has been confirmed. Too soon sometimes, as was indicated by those who, after first having applied for CVS, preferred termination of pregnancy for psychosocial reasons, or who declined antenatal diagnosis altogether.

Also, a decision made early in pregnancy for 16th week amniocentesis instead

of 10th week CVS, tends to underestimate the long wait for the amniocentesis result (±3 weeks).

Informing couples about a normal result in writing by mail might be preferable to informing them by telephone. If they so desire, they can be informed about the sex of their child by their attending physician or midwife later on.

9. The recent 40% increase in the cost of (the clinical part of) CVS reflects the extra time needed for counselling compared with the period when amniocentesis was the only option and the extra time and manpower spent at sampling. However, it does not reflect the 'medicalization' of a substantial 'drop-out' group consisting of women with a pregnancy of doubtful viability diagnosed at intake that has to be confirmed by follow-up ultrasound examination(s), as well as of women with a clearly non-viable pregnancy which is subsequently 'taken care of' by the gynaecologist, instead of by nature itself.

8 SAMENVATTING

In de eerste twee jaar na onze start in november 1984 met de chorion villus biopsie (CVB, ook wel de 'vlokkentest' genoemd), kregen wij 427 aanmeldingen uit Noord Nederland voor deze vorm van antenatale diagnostiek, te weten het onderzoek naar foetale chromosomale afwijkingen.

Hoewel het gemiddelde opkomstpercentage voor de drie noordelijke provincies gelijk was aan het landelijke gemiddelde van 30%, liepen de percentages voor Groningen, Friesland en Drenthe onderling nogal uiteen. Tevens vond een verschuiving in het verwijspatroon plaats van de tweede- naar de eerste lijns gezondheidszorg. Bij 216 zwangeren werd een biopsie verricht. Bij 211 zwangeren werd de test om uiteenlopende redenen niet uitgevoerd. (hoofdstuk 2 en 3)

De ingreep werd transcervicaal en onder gelijktijdige echoscopische controle uitgevoerd bij een zwangerschapsduur van ongeveer 10 weken. (hoofdstuk 4)

De CVB mislukte bij 15 vrouwen (6.9%): bij 12 van hen mislukte de biopsie zelf omdat geen of ongeschikte vlokken werden verkregen, en bij de resterende drie zwangeren mislukte de karyotypering van de vlokken terwijl deze van goede kwaliteit leken te zijn.

Bij 10 vrouwen was de uitslag abnormaal (5%): 3x trisomie 21, 3x mozaïek, 2x triploïdie, 1x 'deletie-16' en 1x polyploïdie.

Naast terecht positieve- kwamen ook fout-positieve uitslagen voor. Tien zwangerschappen eindigden onbedoeld in een miskraam of een voortijdige bevalling (4.8%) respectievelijk in zwangerschapsweek 12, 13, 16, 17(3x), 18, 20, 22 en 26. Dit laatste kind overleefde het en is gezond.

De meest geuite klacht na de CVB was licht vaginaal bloedverlies gedurende een paar dagen na de ingreep.

Bij het schrijven van dit proefschrift waren alle voortgaande zwanger- schappen afgelopen, de laatste op 13 juni 1987. (sectie 5.1)

In onze studiegroep was het niet exact bekende abortus risico van de vlokkentest de belangrijkste reden om aan de vruchtwaterprik de voorkeur te geven. Een andere belangrijke groep uitvallers werd gevormd door die vrouwen van wie de zwangerschap vóór de test in een miskraam eindigde. (sectie 5.2)

Het verschil in concentraties van het alfa-fetoproteïne in het maternale serum (MSAFP) vóór en na de CVB en bij 16 weken zwangerschapsduur, wees op een aanzienlijke, zij het tijdelijke foeto-maternale transfusie tengevolge van de ingreep. Tevens bleek een sterk verband te bestaan tussen lage MSAFP spiegels en foetale trisomie 21. (sectie 6.1)

Foetale bewegingspatronen, geregistreerd vlak vóór en meteen na de CVB verschilden nauwelijks, zelfs niet indien de ingreep tamelijk traumatisch was geweest. (sectie 6.2)

Er kon geen verband worden gelegd tussen enerzijds een aantal belangrijke karakteristieken van de ingreep (zoals het gewicht van de vlokken, al dan niet met bloedbijmenging, het aantal canule-inserties e.d.), en anderzijds het verdere zwangerschapsbeloop en placentapathologie. (sectie 6.3)

Bepaalde aspecten van het gedrag en de ervaringen van zwangeren met betrekking tot de keuze 'vlokkentest of vruchtwaterpunctie?' waren toch opvallend. Zo had bijvoorbeeld de 'vruchtwatergroep' zich duidelijk geen goed idee gevormd over de gevoelens die kunnen optreden wanneer men laat in de zwangerschap met de mogelijkheid van een zwangerschapsafbreking wordt geconfronteerd. Ook hadden sommigen de uitslag liever schriftelijk dan telefonisch vernomen. (sectie 6.4)

Over het algemeen blijkt de eerste trimester chorion villus biopsie een welkome aanvulling te zijn op de tweede trimester amniocentese voor de diagnostiek van foetale chromosomale afwijkingen gezien het aantal vrouwen dat voor een vlokkentest kiest. De voordelen van een test zo vroeg in de zwangerschap wegen zwaarder dan een aantal duidelijke nadelen. Van die nadelen lijken bepaalde problemen overigens kleiner te zijn dan aanvankelijk werd gedacht (in het bijzonder het abortus risico), maar andere juist groter (bijvoorbeeld de discongruente uitslagen).

Hoe de CVB zich in voor- en nadelen werkelijk verhoudt tot de amniocentese zou hebben moeten blijken uit gerandomiseerd onderzoek tussen deze beide methoden van antenatale diagnostiek. Daarvoor is het in Nederland nu te laat.

9 REFERENCES

Acosta-Sison H. Diagnosis of hydatidiform mole. Obstet Gynecol 1958; 12: 205-8.

Adinolfi M. Personal communication. International Symposium First Trimester Genetic Diagnosis and Therapy, Chicago, 1987.

Alvarez H. Diagnosis of hydatidiform mole by transabdominal placental biopsy. Am J Obstet Gynecol 1966; 95: 538.

Anonymus. De oudere zwangere dient bijzondere informatie ook duidelijk geboden te worden. Ned Tijdschr Geneeskd 1986; 130: 457.

Anonymus. Gynaecoloog kwijt zich onvoldoende van zijn informatieplicht; maatregel van waarschuwing. Med Contact 1986; 41: 928-9.

Anshan Iron and Steel Company. Department of Obstetrics and Gynaecology, Tietung Hospital. Fetal sex prediction by sex chromatin of chorionic villi cells during early pregnancy. Chin Med J 1975; 1: 117-26.

Arendzen JH, Huisjes HJ. A simple method for prenatal determination of sex. Acta Cytol 1971; 15: 316-7.

Bakker E, Goor N, Wrogemann K, Kunkel LM, Fenton WA, Majoor-Krakauer D, Jahoda MGJ, Ommen GJB van, Hofker MH, Mandel JL, Davies KE, Willard HF, Sandkuyl L, Essen AJ van, Sachs ES, Pearson PL. Prenatal diagnosis and carrier detection of Duchenne muscular dystrophy with closely linked RFLP's. Lancet 1985; i: 655-8.

Barkai G, Shaki R, Pariente C, Goldman B. First trimester alpha-fetoprotein levels in normal and chromosomally abnormal pregnancies. Lancet 1987; ii: 389.

Bennebroek Gravenhorst J. De 'vlokkentest', veilig, snel en betrouwbaar (Ingezonden). Ned Tijdschr Geneeskd 1986; 130: 37.

Bennebroek Gravenhorst J, Overbeek MAM, van Dijk BA. Problemen bij de anti-rhesus(D)-immunoprofylaxe. Ned Tijdschr Geneeskd 1984; 128: 1005-9.

Bevis DCA. Composition of liquor amnii in haemolytic disease of the newborn. Lancet 1950; ii: 443.

Bevis DCA. The antenatal prediction of haemolytic disease of the newborn. Lancet 1952; i: 395-8.

Bevis DCA. Blood pigments in haemolytic disease of the newborn. J Obstet Gynaecol Brit Emp 1956; 63: 68-75.

Blakemore KJ, Mahoney MJ, Hobbins JC. Infection and chorionic villus sampling. Lancet 1985; ii: 339.

Blakemore KJ, Mahoney MJ. Chorionic villus sampling. In: Milunsky A ed. Genetic Disorders and the Fetus. New York: Plenum Press, 1986: 625-60.

Boogert A, Mantingh A, Visser GHA. The immediate effects of chorionic villus sampling on fetal movements. Am J Obstet Gynecol 1987; 157: 137-9.

Boyd PA. Placenta and umbilical cord. In: Keeling JW ed. Fetal and neonatal pathology. London, Berlin, Heidelberg, New York, Paris, Tokyo: Springer Verlag 1987: 45-76.

Brambati B, Simoni G. Diagnosis of fetal trisomy 21 in first trimester of pregnancy. Lancet 1983; i: 586.

Brambati B, Simoni G, Danesino C, Oldrini A, Ferrazi E, Romitti L, Terzoli G, Rosella F, Ferrari M, Fraccaro M. First trimester fetal diagnosis of genetic disorders: clinical evaluation of 250 cases. J Med Genet 1985; 22: 92-9.

Brambati B, Varotto F. Infection and chorionic villus sampling. Lancet 1985; ii: 609.

Brambati B, Simoni G, Bonacchi I, Piceni L. Fetal chromosomal aneuploidies and maternal serum alpha-fetoprotein levels in first trimester. Lancet 1986; ii: 165-6.

Brambati B, Oldrini A, Ferazzi E, Lanzani A. Chorionic villus sampling: an analysis of the obstetric experience of 1000 cases. Prenat Diagn 1987; 7: 157-69.

Breed A, Mantingh A, Govaerts L, Boogert A, Laurini R, Anders G. Structural chromosomal aberration confined to the placenta. The Second International Conference on Chorionic Villi Sampling and Early Prenatal Diagnosis. Birmingham, 1985.

Breed A, Mantingh A, Govaerts L, Boogert A, Anders G, Laurini R. Abnormal karyotype, not confirmed in a subsequently aborted fetus. Prenat Diagn 1986; 6: 375-7.

Breed ASPM, Mantingh A, Kloosterman MD, Govaerts LCP, Beekhuis JR, Anders GJPA. Predictive value of chromosomal diagnosis on chorionic villus material, 350 cases. Third International Conference Villi Sampling and Early Prenatal Diagnosis. Strasbourg, 1986. (abstract no.16)
Brock DJH, Sutcliffe RG. α-Fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. Lancet 1972; ii: 197-9.
Brock DJH, Bolton AE, Monaghan JM. Prenatal diagnosis of anencephaly through maternal serum alpha-fetoprotein measurement. Lancet 1973; ii: 923-4.
Brock DJH. Early Diagnosis of Fetal Defects. Current Reviews in Obstetrics and Gynaecology. Churchill-Livingstone, Edinburgh, 1982. (Lind T, series editor)

Callen DF, Korban G, Dawson G, Krumins EJM, Eichenbaum S, Purvis-Smith S, Smith A, Dulk G den, Martin N. Extra-embryonic/fetal karyotypic discordance during diagnostic chorionic villus sampling. International Symposium First Trimester Genetic Diagnosis and Therapy, Chicago, 1987. (abstract no.11)
Campbell AV. Ethical issues in prenatal diagnosis. Br Med J 1984; 228: 1633-4.
Campbell S, Johnstone FD, Holt EM, May P. Anencephaly. Early Ultrasonic diagnosis and management. Lancet 1972; ii: 1226-7.
Campbell S. Diagnosis of fetal abnormalities by ultrasound. In: Milunsky A ed. Genetic Disorders and the Fetus. New York: Plenum Press, 1979: 431-67.
Christiaens GCML, Kriek M, Korenromp M. Prenatale Diagnostiek met behulp van amniocentese. In: Kloosterman ed. Prenatale Diagnostiek in de eerste helft van de zwangerschap. Apeldoorn: Medicom Europe, 1987: 26-41.
Cordone M, Lituania M, Passamonti U, Couco C, Pezollo A. Feto-placental discrepancies: a possible role of first trimester amniocentesis. International Symposium First Trimester Genetic Diagnosis and Therapy, Chicago, 1987. (abstract no.7)
Covone AE, Mutton D, Johnson PM, Adinolfi M. Trophoblast cells in peripheral blood from pregnant women. Lancet 1984; ii: 841-3.
Covone AE, Johnson PM. Identification of trophoblast in chorionic villi biopsy samples. Hum Genet 1986; 72: 172-3.
Crandell BF, Lebherz TB, Tabsh K. Maternal age and amniocentesis: should this be lowered to 30 years? Prenat Diagn 1986; 6: 237-42.
Cuckle HS, Wald NJ, Lindenbaum RH. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. Lancet 1984; i: 926-9.

Daffos F, Capella-Pavlovski M, Forestier F. Fetal bloodsampling via the umbilical cord using a needle guided by ultrasound. Prenat Diagn 1983; 3: 271-7.
Donald I, Mac-Vicar J, Brown TG. Investigation of abdominal masses by pulsed ultrasound. Lancet 1958, i: 1188-95.
Dumez Y, Goossens M, Boué J, Poenaru L, Dommergues M, Henrion R. Chorionic villi sampling using rigid forceps under ultrasound control. In: Fraccaro M, Simoni G, Brambati B eds. First trimester fetal diagnosis. Berlin, Heidelberg, New York, Tokyo: Springer Verlag 1985: 39-45.

Editorial. Vaginal bleeding in early pregnancy. Br Med J 1980; 281: 470.
Editorial. Can first-trimester fetal diagnosis be reliably evaluated? Lancet 1985; i: 735-6.
Eichenbaum SZ, Krumins EJ, Fortune DW, Duke J. False negative finding on chorionic villus sampling. Lancet 1986; ii: 391.
Evans EP, Burtenshaw MD, Ford CE. Chromosomes of mouse embryos and newborn young: Preparation from membranes and tail tips. Stain Technol 1972; 47: 229-34.

Farrant W. Stress after amniocentesis for high serum alpha-fetoprotein concentrations. Br Med J 1980; ii: 452.
Fletcher JC. Ethical issues in clinical trials of first trimester prenatal diagnosis. In: Brambati B, Simoni G, Fabro S eds. Chorionic villus sampling. Fetal diagnosis of genetic diseases in the first trimester. New York, Basel: Marcel Dekker, Inc. 1986: 275-97.
Ford CE, Hamerton JL. The chromosomes of man. Nature 1956; 178: 1020-3.
Fuchs F, Riis P. Antenatal sex determination. Nature 1956; 177: 330.

Galjaard H. De 'vlokkentest', veilig, snel en betrouwbaar. Ned Tijdschr Geneeskd 1985; 129: 1955-7.
Galjaard H. De 'vlokkentest', veilig, snel en betrouwbaar (Ingezonden). Ned Tijdschr Geneeskd 1986; 130: 37.
Garden AS, Reid G, Benzie RJ. Infection and chorionic villus sampling. Lancet 1985; i: 1270.
Gibbs DA, Crawfurd M d'A, Headhouse-Benson CM, McFadyen IR, Muinck Keizer EE de, Wilson TM, Farrant PH. First trimester diagnosis of Lesh-Nyhan syndrome. Lancet 1984; ii: 1180.
Gilmore DM, McNay MB. Spontaneous fetal loss rate in early pregnancy. Lancet 1985; i: 107.
Golbus MS, Kan YW, Naglich-Craig M. Fetal blood sampling in midtrimester pregnancies. Am J Obstet Gynecol 1976; 124: 653-5.
Goldberg MF, Chen ATL, Young WA, Reidy JA. First trimester fetal chromosome diagnosis using endocervical lavage: A negative evaluation. Am J Obstet Gynecol 1980; 138: 436-40.
Goodfellow CF, Taylor PV. Extraction and identification of trophoblast cells circulating in peripheral blood during pregnancy. Br J Obstet Gynaecol 1982; 89: 65-8.
Goossens M, Dumez Y, Kaplan L, Lupker M, Chabret C, Henrion R, Rosa J. Prenatal diagnosis of sickle-cell anaemia in the first trimester of pregnancy. N Engl J Med 1983; 309: 831-3.
Grebner EE. Wapner RJ, Barr MA, Jackson LG. Prenatal Tay-Sachs diagnosis by chorionic villus sampling. Lancet 1983; ii: 286-7.
Grosse-Wilde H, Miny W, Pawlowitzki IH. Prenatal diagnosis of paternity by HLA typing of cultured cells after CVS. Third International Conference Chorionic Villi Sampling and Early Prenatal Diagnosis, Strasbourg, 1986. (abstract no.11)
Gustavii B. First-trimester chromosomal analysis of chorionic villi obtained by direct vision technique. Lancet 1983; ii: 507-8.
Gustavii B. Chorion biopsy and miscarriage in first trimester. Lancet 1984; i: 562.

Hagard S, Carter FA. Preventing the birth of infants with Down'ssyndrome: a cost-benefit analysis. Br Med J 1976; i: 753-6.
Hahnemann N, Mohr J. Genetic diagnosis in the embryo by means of biopsy from extra-embryonic membranes. Bull Eur Soc Hum Genet 1968; 2: 23-29.
Hahnemann N, Mohr J. Antenatal foetal diagnosis in genetic disease. Bull Eur Soc Hum Genet 1969; 3: 47-54.
Hahnemann N. Early prenatal diagnosis: a study of biopsy techniques and cell culturing from extra-embryonic membranes. Clin Genet 1974; 6: 294-306.
Hahnemann N. Personal communication. Third International Conference Chorionic Villi Sampling and Early Prenatal Diagnosis. Strasbourg, 1986.
Hahnemann N. Personal Communication. International Symposium First Trimester Genetic Diagnosis and Therapy. Chicago, 1987.
Han Anguo, Zhou Bingru, Wang Hong. Long term follow-up results after aspiration of chorionic villi during early pregnancy. In: Fraccaro M, Simoni G, Brambati B eds. First trimester fetal diagnosis. Berlin, Heidelberg, New York, Tokyo: Springer Verlag 1985: 1-6.
Hanson FW, Zorn EM, Tennant FR, Marianos S, Samuels S. Amniocentesis before 15 weeks gestation: Outcome, risks, and technical problems. Am J Obstet Gynecol 1987; 156: 1524-31.
Harlap S, Shiono PH, Ramcharan S. A life table of spontaneous abortions and the effects of age, parity and other variables. In: Porter IH, Hook EB eds. Embryonic and fetal death. New York: Academic Press, 1980: 145-58.
Haveman MJ. Prognoses over het aantal geestelijk gehandicapten anno 2000. T Soc Gezondheidsz 1985; 63: 718-26.
Hecht F, Hecht BK, Bixenman HA. Caution about chorionic villus sampling in the first trimester. N. Engl J Med 1984; 310: 1388.
Hecht F, Kaiser McCaw Hecht B. Fetal loss after chorionic villus sampling (CVS) in small and large centres. Prenat Diagn 1985; 5: 167.
Hobbins JC, Mahoney MJ. In utero diagnosis of haemoglobinopathies. Technic for obtaining fetal blood. N Engl J Med 1974; 290: 1065-7.
Hobbins JC, Grannum PA, Romero R, Reece EA, Mahoney MJ. Percutaneous umbilical bloodsampling. Am J Obstet Gynecol 1985; 152: 1-6.
Hogge WA, Schonberg SA, Golbus MS. Prenatal diagnosis by chorionic villus sampling: lessons of the first 600 cases. Prenat Diagn 1985; 5: 393-400.

Holzgreve W, Miny P, Ritzerfield W. Bacterial contamination of the catheter after transcervical CVS. Third International Conference Chorionic Villi Sampling and Early Prenatal Diagnosis, Strasbourg, 1986. (abstract no.7)
Horwell DH, Heaton DE, Old JM. Diagnosis of haemoglobinopathies using cultured chorionic villus cells. Lancet 1985; ii: 613-4.
Huether C. Projection of Down's syndrome births in the United States 1979-2000, and the potential effects of prenatal diagnosis. Am J Publ Health 1983; 73: 1186-9.
Huisjes HJ, Mantingh A. Eerste ervaringen met prenatale diagnostiek van aangeboren afwijkingen in de 8e - 9e week van de zwangerschap door onderzoek van chorionvlokken (Ingezonden). Ned Tijdschr Geneeskd 1984; 128: 1161.

Jaarverslag Antenatale Diagnostiek 1987. Kliniek voor Obstetrie en Gyaecologie, Academisch Ziekenhuis, Groningen.
Jackson LG, Wapner RJ, Grebner EE, Barr MA. Fetal genetic diagnosis by chorionic villus sampling. In: Human prenatal diagnosis. Filkins K, Russo JF eds. New York, Basel: Marcel Dekker, Inc. 1985.: 285-310.
Jackson LG. CVS Newsl (Jefferson Medical College, Philadelphia): Apr 85/10; Jan 86/15; Mar 86/16; May 86/17; May 87/21; Nov 87/23.
Jackson LG. Personal Communication. International Symposium First Trimester Genetic Diagnosis and Therapy. Chicago, 1987.
Jacobson CB, Barter RH. Intrauterine diagnosis and management of genetic defects. Am J Obstet Gynecol 1967; 99: 796-807.
Jahoda M, Sachs ES, Kleyer WJ, Drogendijk AC, Galjaard H. Eerste ervaringen met prenatale diagnostiek van aangeboren afwijkingen in de 8e - 9e week van de zwangerschap door onderzoek van chorionvlokken. Ned Tijdschr Geneeskd 1984; 128: 436-42.
Jahoda MGJ. Personal Communication. Third International Conference Chorionic Villi Sampling and Early Prenatal Diagnosis. Strasbourg, 1986.
Jahoda MGJ, Pijpers L, Vosters RPL, Wladimiroff JW, Reuss A, Sachs ES. Role of maternal age assessment of risk of abortion after prenatal diagnosis during first trimester. Br Med J 1987; 295: 1237.
Kanhai HHH, Bennebroek Gravenhorst J, Veer MB van 't, Maas CJ, Beverstock GC, Bernini LF. Chorionic biopsy in management of severe rhesus isoimmunisation. Lancet 1984; ii: 157-8.
Kanhai HHH, Veer MB van 't, Bernini LF, Beverstock GC, Bennebroek Gravenhorst J. Bepaling van de resusfactor in het eerste trimester van de zwangerschap met behulp van chorionvillibiopsie. Ned Tijdschr Geneeskd 1985; 129: 2237.
Kaplan L, Dumez Y, Goossens M. A method for fetal tissue sampling by chorionbiopsy: A new approach to first trimester prenatal detection of abnormal genes. I.R.C.S. Med Sci 1983; 11: 85-6.
Katz-Rothman B. The tentative pregnancy: prenatal diagnosis and the future of motherhood. Viking Penguin, New York, 1986.
Kazy Z, Rozovsky IS, Bakharev VA. Chorionbiopsy in early pregnancy: a method of early prenatal diagnosis for inherited disorders. Prenat Diagn 1982; 2: 39-45.
Kearney L. Fragile X in chorionic villi. The Second International Conference on Chorionic Villus Sampling and Early Prenatal Diagnosis, Birmingham, 1985 (abstract no. 21).
Keppels M, Velde A S van der, Tijmstra Tj, Mantingh A. Vlokkentest of vruchtwaterpunctie? Een onderzoek naar het keuzegedrag en de ervaringen van zwangeren. Med Contact 1987; 42: 462-4.
Kleyer WJ, Diggelen OP van, Janse HC, Galjaard H, Dumez Y, Boué J. First trimester diagnosis of Hunter syndrome on chorionic villi. Lancet 1984; ii: 472.
Kleyer WJ, Janse HC, Vosters RPL, Niermeyer MF, Kamp JJP van de. First-trimester diagnosis of mucopolysaccharidosis III A (Sanfilippo A disease). N Engl J Med 1986; 314: 185-6.
Kleyer WJ, Janse HC, Diggelen OP van, Macek M, Hajek Z, Gillett MG, Holton JB. First-trimester diagnosis of galactosaemia. Lancet 1986; i: 748.
Kleyer WJ, Hussaarts-Odijk LM, Sachs ES, Jahoda MGJ, Niermeyer MF. Prenatal Diagnosis of Fabry's disease by direct analysis of chorionic villi. Prenat Diagn 1987; 7: 283-7.
Kloosterman MD, Christiaens GCML. De 'vlokkentest', veilig, snel, en betrouwbaar. (Ingezonden). Ned Tijdschr Geneeskd 1986; 130: 36-7.
Kloosterman MD. Het abortusprobleem. In: Kloosterman ed. Prenatale diagnostiek in de eerste helft van de zwangerschap. Apeldoorn: Medicom Europe 1987: 94-100.

Kloosterman MD. Personal communication Symposium Antenatal Diagnosis, Dutch Society of Obstetricians and Gynaecologists, 1988.
Kullander S, Sandahl B. Fetal chromosome analysis after transcervical placental biopsies in early pregnancy. Acta Obstet Gynecol Scand 1973; 52: 355-9.

Lachman E, Hingley SM, Bates G, Ward AM, Stewart CR, Duncan SLB. Detection and measurement of fetomaternal hemorrhage: serum alpha-fetoprotein and the Kleihauer technique. Br Med J 1977; 1: 1377-9.
Lejeune J, Turpin R, Gautier M. Le mongolisme. Premier example d'aberration autosomique humaine. Ann Genet 1959; 1: 41.
Lele AS, Carmody PK, Hurd ME, O'Leary LA. Fetomaternal bleeding following diagnostic amniocentesis. Obstet Gynecol 1982; 60: 60-4.
Leschot NJ. Resultaten van prenatale diagnostiek bij 350 zwangeren in de 10e week van de zwangerschap (Ingezonden). Ned Tijdschr Geneeskd 1986; 130: 36.
Leschot NJ, Wolf H, Verjaal M, Prooyen-Knegt LC van, Boer EG de, Kanhai HHH, Christiaens GCML. Chorionic villi sampling: cytogenetic and clinical findings in 500 pregnancies. Br Med J 1987; 295: 407-10.
Lilford R, Maxwell D, Coleman D, Czepulowsky B, Heaton D. Diagnosis, four hours after chorion biopsy, of female fetus in pregnancy at risk of Duchenne muscular dystrophy. Lancet 1983; ii: 1491.
Liu DTY, Mitchell J, Johnson J, Wass DM. Trophoblast sampling by blind transcervical aspiration. Br J Obstet Gynaecol 1983; 90: 1119-23.
Löfberg L, Iosif CS, Edvall H, Gustavii B. Direct vision sampling of chorionic villi during extra-amniotic instillation of physiologic saline solution: effects on intrauterine pressure and fetal heart activity. Am J Obstet Gynecol 1985; 152: 591-2.

Mantingh A, Breed ASPM, Boogert A, Govaerts LCP. The other side of the CVS coin. Eur J Epidemiol 1986a; 2: 324-5.
Mantingh A, Breed ASPM, Boogert A, Govaerts LCP. Voor en na de vlokkentest. Ned Tijdschr Geneeskd 1986b; 30: 1327-9.
Mariona FG, Bhatia R, Syner FN, Koppitch F. Chorionic villi sampling changes maternal serum alpha-fetoprotein. Prenat Diagn 1986; 6: 69-73.
Martin AO, Sherman E, Rosinsky B, Bombard AT, Simpson JL. False negative finding on chorionic villus sampling. Lancet 1986; ii: 391-2.
McFadyen IR, Taylor-Robinson D, Furr PM, Boustouller YL. Infection and chorionic villus sampling. Lancet 1985; ii: 610.
Meeuwissen JHJM. Informatieplicht. Bijzondere informatie aan oudere zwangere (Ingezonden). Med Contact 1986; 41: 973.
Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol 1984; 148: 886-91.
Mikkelsen M. Cytogenetic findings in first trimester chorionic villi biopsies: a collaborative study. In: Fraccaro M, Simoni G, Brambati B eds. First trimester fetal diagnosis. Berlin, Heidelberg, New York, Tokyo: Springer Verlag 1985: 109-20.
Mikkelsen M. Personal Communication. Third International Conference Chorionic Villi Sampling and Early Prenatal Diagnosis. Strasbourg, 1986.
Milunsky A. Personal communication. The Second International Conference on Chorionic Villus Sampling and Early Prenatal Diagnosis, Birmingham, 1985.
Milunsky A. Personal communication. International Symposium First Trimester Genetic Diagnosis and Therapy, Chicago, 1987.
Modell B. Chorionic Villus Sampling. Evaluating Safety and Efficacy. Lancet 1985; i: 737-40.
Mohr J. Fetal genetic diagnosis: development of techniques for early sampling of foetal cells. Acta Pathol Microbiol Scand 1968; 73: 73-7.
Mollison PL. Suppression of Rh-isoimmunization by passively administered anti-Rh. Br J Haematol 1968; 14: 1-4.
Muggah HF, D'Alton ME, Hunter AGW. Chorionic villus sampling followed by genetic amniocentesis and septic shock. Lancet 1987; i: 867-8.

Nadler HL. Antenatal detection of hereditary disorders. Paediatrics 1968; 42: 912-8.
Netherlands Central Bureau of Statistics, The Hague. Monthly bulletin of Population Statistics, 1987.
Niazi M, Coleman DV, Loeffler FE. Trophoblast sampling in early pregnancy. Culture of rapidly dividing cells from immature placental villi. Br J Obstet Gynaecol 1981; 88: 1081-5.
NICHD National Registry for Amniocentesis Study Group. Midtrimester amniocentesis for prenatal diagnosis: safety and accuracy. JAMA 1976; 236: 1471-6.
Nicolaides KH, Rodeck CH, Mibashan RS. Obstetric management and diagnosis of haematological disease in the fetus. Clinics in Haematology 1985; 14: 775-805.

Old JM, Ward RHT, Petrou M, Karagözlü F, Modell B, Weatherall DJ. First trimester fetal diagnosis for haemoglobinopathies: three cases. Lancet 1982; ii: 1413-6.

Pergament E, Ginsberg N, Verlinsky Y, Cadkin A, Chu A, Trinka L. Prenatal Tay-Sachs diagnosis by chorionic villus sampling. Lancet 1983; ii: 26.
Perry TB, Vekemans MJJ. Lippman A, Hamilton EF, Fournier PJR. Chorionic villi sampling: clinical experience, immediate complications, and patient attitudes. Am J Obstet Gynecol 1985; 151: 161-6.
Pijpers L, Reuss A, Jahoda MGJ, Sachs ES, Wladimiroff JW. De 'vlokkentest' (chorionvillus-biopsie) in het 2e en 3e trimester van de zwangerschap. Ned Tijdschr Geneeskd 1988; 132: 765-7.

Report to the Medical Research Council by their Working Party on Amniocentesis. An assessment of the hazards of amniocentesis. Br J Obstet Gynaecol 1978; 85: suppl 2, 1-41.
Rhine SA, Caim JL, Leary RE, Palmer CG, Thompson JF. Prenatal sex detection with endocervical smears: Successful results utilizing Y-body fluorescence. Am J Obstet Gynecol 1975; 122: 155.
Rhine SA, Milunsky A. Utilization of trophoblast for early prenatal diagnosis. In: Milunsky A ed. Genetic disorders and the fetus. New York: Plenum Press 1979: 527-39.
Rodeck CH, Morsman JM, Gosden CM, Gosden JR. Development of an improved technique for first trimester microsampling of chorion. Br J Obstet Gynaecol 1983; 90: 1113-8.

Sachs ES, Jahoda MGJ, Vosters RPL, Kleyer WJ, Galjaard H. Resultaten van prenatale diagnostiek by 350 zwangeren in de 10e week van de zwangerschap. Ned Tijdschr Geneeskd 1985; 129: 1968-74.
Schmidt W, Garoff L, Heberling D, Zaloumis M, Cseh I, Haller U, Kubli F. Uberwachung der fetalen Bewegungsaktivität mit Real-Time-Ultraschall und deren Bedeutung für den Schwangerschafsverlauf. Geburtsch Frauenheilk 1981; 41: 601-6.
Scrimgeour JB. Other techniques for antenatal diagnosis. In: Emery AEH ed. Antenatal diagnosis of genetic disease. Edinburgh: Churchill Livingstone 1973: 40-57.
Scrimgeour JB. Personal Communication. The Second International Conference on Chorionic Villi Sampling and Early Prenatal Diagnosis. Birmingham, 1985.
Selypes A, Lorencz R. A noninvasive method for determination of the sex and karyotype of the fetus from the maternal blood. Hum Genet 1988; 79: 357-9.
Serr DM, Sachs L, Danon M. Diagnosis of sex before birth using cells from the amniotic fluid. Bull Res Counc Isr 1955; 5B: 137.
Shettles LB. Nuclear morphology of cells in human amniotic fluid in relation to the sex of the infant. Am J Obstet Gynecol 1956; 71: 834-8.
Simoni G, Brambati B, Danesino C, Rosella F, Terzoli GL, Ferrari M, Fraccaro M. Efficient direct chromosome analysis and enzyme determinations from chorionic villi samples in the first trimester of pregnancy. Hum Genet 1983; 63: 349-57.
Simoni G, Brambati B, Danesino C, Terzoli GL, Romitti L, Rosella F, Fraccaro M. Diagnostic application of first trimester trophoblast sampling in 100 pregnancies. Hum Genet 1984; 66: 252-9.
Simoni G, Gimelli G, Cuoco G, Romitti L, Terzoli G, Guerneri S, Rossella F, Pescetto L, Pezzolo A, Porta S, Brambati B, Porro E, Fraccarro M. First trimester karyotyping: one thousand diagnoses. Hum Genet 1986; 72: 203-9.
Simpson GF, Sun NH, Golbus MS. Changes in maternal serum alpha-fetoprotein levels associated with intrauterine genetic diagnosis procedures. Prenat Diagn 1984; 4: 207-11.

Simpson NE, Dallaire L, Miller JR, Siminovich L, Hamerton JL, Miller J, McKeen C. Prenatal diagnosis of genetic disease in Canada, report of a collaborative study. Can Med Assoc J 1976; 115: 739-46.
Singh JR. Increased frequency of selective female foeticide - an additional risk of chorionic villi studies. The Second International Conference on Chorionic Villus Sampling and Early Prenatal Diagnosis, Birmingham 1985. (abstract no.3)
Smidt-Jensen S, Hahnemann N. Transabdominal fine needle biopsy from chorionic villi in the first trimester. Prenat Diagn 1984; 4: 163-9.
Smidt-Jensen S, Hahnemann N, Hariri J, Jensen PKA, Therkelsen AJ. Transabdominal chorionic villi sampling for first trimester diagnosis. First 26 pregnancies followed to term. Prenat Diagn 1986; 6: 125-32.
Smithies O. Personal communication. International Symposium First Trimester Genetic Diagnosis and Therapy, Chicago, 1987.
Steele MW, Breg WR Jr. Chromosome analysis of human amniotic fluid cells. Lancet 1966; i: 383-5.
Stichting Erfelijkheidsvoorlichting Annual Reports 1979-1981, 1982, 1983, and 1984.
Szabó J, Herczeg J, Thurzo L, Szemere G. Karyotyping from uncultured human trophoblast in the first trimester of pregnancy. Obstet Gynecol 1984; 6: 807-10.

Tabor A, Larson SO, Nielsen J, Nielsen Joh, Philip J, Pilgaard B, Videbech P, Nørgaard-Pedersen B. Screening for Down's syndrome using an iso-risk curve based on maternal age and serum alpha-fetoprotein level. Br J Obstet Gynaec 1987; 94: 636-42.
Tabsh KMA, Lebherz TB, Crandall BF. Risks of prophylactic anti-D immunoglobulin after second trimester amniocentesis. Am J Obstet Gynecol 1984; 149: 225-6.
Therkelsen AJ, Jensen PKA, Hertz JM, Smidt-Jensen S, Hahneman N. Prenatal cytogenetic diagnosis after transabdominal chorionic villus sampling in the first trimester. Prenat Diagn 1988; 8: 19-31.
Thiede HA, Creasman WT, Metcalfe S. Antenatal analysis of the human chromosomes. Am J Obstet Gynecol 1966; 94: 589-90.
Thomassen-Brepols LJ, Duivenvoorden HJ, Verhage F, Galjaard H. Prenataal onderzoek bij vrouwen van 38 jaar en ouder. Med Contact 1983; 52: 1637-40.
Thomassen-Brepols LJ. Psychosociale aspecten van prenatale diagnostiek. Academic thesis. Rotterdam: Pasmans BV, 1985.
Tijmstra Tj, Huisjes HJ, Kate LP ten. Vruchtwateronderzoek. Gebruik prenatale chromosomale diagnostiek door vrouwen van 38 jaar en ouder. Med Contact 1982; 37: 765-7.
Tijmstra Tj. Het imperatieve karakter van medische technologie en de betekenis van 'geanticipeerde beslissingsspijt'. Ned Tijdschr Geneeskd 1987; 131: 1128-31.
Tjio HJ, Levan A. The chromosome number of man. Hereditas 1956; 42: 1-6.

Visser GHA, Laurini RN, Vries JIP de, Bekedam DJ, Prechtl HFR. Abnormal motor behaviour in anencephalic fetuses. Early Hum Dev 1985; 12: 173-82.
Visser GHA, Bekedam DJ, Mulder EJH, Ballegooie E van. Delayed emergence of fetal behaviour in type-1-diabetic women. Early Hum Dev 1985; 12: 167-72.
Vries JIP de, Visser GHA, Prechtl HFR. The emergence of fetal behaviour. 1. Qualitative aspects. Early Hum Dev 1982; 7: 301-22.

Ward RHT, Modell B, Petrou M, Karagözlü F, Douratsos E. A method of chorionic villus sampling in the first trimester of pregnancy under real-time ultrasonic guidance. Br Med J 1983; 286: 1542-4.
Warren RC, Butler J, Morsman JM, McKenzie C, Rodeck CH. Does chorionic villus sampling cause fetomaternal haemorrhage? Lancet 1985; i: 691.
Wass D, Benett MJ. Infection and chorionic villus sampling. Lancet 1985; ii: 338-9.
West JD, Gosden JR, Angell RR, Hastie ND, Thatcher SS, Glasier AF, Baird DT. Sexing the human pre-embryo by DNA-DNA in-situ hybridisation, Lancet 1987; i: 1345-7.
Williams H, Bown CS, Thomas NST, Harper PS, Roberts A, Ppadhyaya, Gosden JR. First trimester fetal sexing in pregnancy at risk for Duchenne muscular dystrophy. Lancet 1983; ii: 586-9.
Williamson R, Eskdale J, Coleman DV, Niazi M, Loeffler FE, Modell B. Direct gene analysis of chorionic villi: A possible technique for first-trimester antenatal diagnosis of haemoglobinopathies. Lancet 1981; ii: 1125-7.

Wyatt PR. The place of chorionic biopsy. Lancet 1983; ii: 847.
Wyatt PR. Chorionic biopsy and increased anxiety. Lancet 1985; ii: 1312-3.

Yamamoto M, Fujimori R, Ito T, Kamimura K, Watanabe G. Chromosome studies in 500 induced abortions. Hum Genet 1975; 29: 9-14.

Zipursky A, Israels LG. The pathogenesis and prevention of Rh immunization. Can Med Assoc J; 1967; 97: 1245-57.

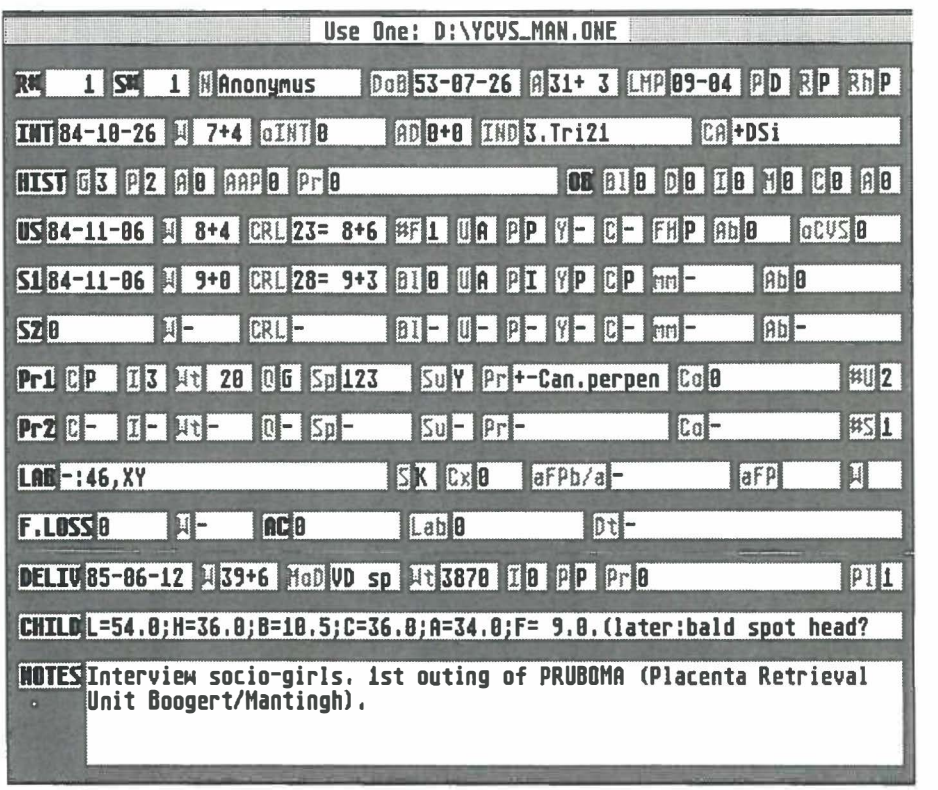
10 STUDY GROUP DATA DEFINITION AND DATABASE STRUCTURE

Data from the filled-out forms I, II, III and IV (see section 11.1) from the total chorionic villus sampling (CVS) intake group (n = 427) were entered into a 100 label, 96 field per record file, using a rather simple, but fast flat-file type database system (DB Master One™ and its upgrade Base Two™, respectively: max. number of fields 100, max. number of characters 3000/field) on a 68000 CPU computersystem (Atari™ STf). These 427 records occupied 230 Kb and were loaded into RAM in toto, enabling fast manipulation of the data.

All data fields were alpha-type and record selections and reporting were done by means of a 3-mode, 3-level searching facility (operators 'equals', 'between' and 'contains', any field against one or two others). Further calculations (totals, rates, statistics etc.) were done by hand.

The record design was created as shown below (the actual screen lay-out differed though: it was tightly cropped in order to reduce the number of screens needed, see screendump Figure 10.1).

Figure 10.1 Filled-out example of database record (screendump).



In the left hand column you will find the labels with short explanatory notes and in the right hand column you see the fields with telegram-style descriptions and the definitions of various data collected, together with explanatory remarks, numbered 1 - 96 inclusive. All fields were filled out in order of the data's relative importance to retain at least the most important ones in long text fields when reporting was carried out by column.

This ultimate design (partly based on Jackson's IBM lay-out, 1985 our departmental Obstetrical Data Information System ('Obdat')) underwent many revisions before it arrived at the set-up below and is still far from perfect. Nevertheless, it provided a head-start as far as the implementation of our computerized routine antenatal diagnosis data collection was concerned (e.g. in avoiding the pittfalls of data definition).

(See list at the end of this chapter for frequently used abbreviations not always explained in the right hand column 'notes' category).

LABELS	FIELDS
1. Record number. (= fixed keyfield)[1]	Entered: Chronologically in order of application for CVS. Note: Same patient entering more than once gets a different number and record.
2. Sampling number. (= number of CVS actually performed)	Note: Repeat sessions carry the same sampling number (see also field no. 38 and no. 75). A = Arnhem L = Leiden
3. Name.	–
4. Date of Birth.	–
5. Age.	Entered as: (completed) Years plus months at time of CVS or AC (≥15 days is rounded up), e.g 38+10. Note 1: – In case of both CVS and AC: time of CVS taken. – In case no procedure done: time of intake used. – In case of no intake: time of application taken. Note 2: – Ultrasound dating not taken as US was not always done. Probable time of conception would have made this data-criterion the same for all, but whichever way, it hardly matters in calculating means etc. It did serve a purpose though in e.g. working out quickly the percentage contribution by age-group in the maternal age indication category (in which e.g. age 35 + 11 at CVS passes as regular maternal age indication (see field no. 14)), defined as having reached 36 years at the time of AC (at 18 weeks at the latest) instead of belonging to the 'anxiety' category.[2]
6. Last monthly period. (= first day of LMP)	Entered as: yy - mm - dd. Note: Date not exactly known within a day or two was considered unreliable and entered as '?'. A last withdrawal bleeding, when I exactly known by date, was entered as such though perhaps 'unreliable'. But subsequent discrepancies will be consistent then and show up in field no. 28, no. 29, no. 39 and no. 40.

[1] Nos 1 - 9 = 'application block' containing data usually obtained at application, and corresponding with form 1 (section 11.1).

[2] See Appendix 11.2 for complete list of indications for antenatal diagnosis.

7. Province.
(= Province of residence)

Entered as:
G (= Groningen)
F (= Friesland)
D (= Drenthe)
O (= Overijssel and N-O Polders)
E (= Elsewhere (not further specified))

8. Referred by.
(= referring person on institution)

Entered as:
P (= General Practitioner)
M (= Midwife)
O (= Obstetrician)
G (= Geneticist)
D (= Dept. Obst.& Gynae. AZG)
S (= Self)
E (= Else (not specified))

Note:
D = our own department.
S = patient's own initiative.
E = e.g. paediatrician, physician, Stimezo, surgeon.

9. Rhesus.
(= rhesusfactor)

Entered as:
N (= Negative)
P (= Positive)

10. Intake.
(= date of intake)[1]

Entered as:
yy - mm - dd

Note:
Regardless whether later follow-up ultrasound was needed to ascertain fetal viability (see field no. 27).

11. Week.
(= gestational age since since 1st day of last monthly period;
= menstrual age based on a 4-wk. cycle)

Entered as:
ww + dd

Note:
By pregnancy calender (like field no. 28, no. 39, no. 83 and no. 88) in the absence of a 'formula field' option in this database.

12. No intake.
(: gives the reasons why, despite application for CVS, intake was not done)

Entered as:
1 (= intercurrent abortion)
2 (= threatened abortion)
3 (= termination of pregnancy, AAP)
4 (= abortionrisk)
5 (= other risk(s))
6 (= incomplete)
7 (= new/unreliable)
8 (= our advice)
9 (= other reasons)
0 (= intake done)
– (= skipped intake)
? (= not known why no intake)

[1] Nos 10 - 37 = 'intake block' containing data usually obtained at intake and corresponding with form II.

Note:
- Up to a maximum of 3 reasons in order of their importance in patient's view (e.g. 746)
- Ad 5: as mentioned in info, see Appendix 11.4
- Ad 6: mostly 'it does not test for NTD', but also 'you can see more with AC' e.g. in case of multiple congenital abnormalities e.c.i.
- Ad 8: e.g. IUCD in situ, gemelli, chemo- and radiotherapy, time needed for further family investigation etc., usually by telephone consultation (room to enter these reasons in field no. 96: up to 256 characters)
- Ad 9: 'declining everything' (both CVS and AC), 'holiday', or 'don't see the point' (see also field no. 37 and specification in field no. 96).

13.Previous Antenatal Diagnosis.
(: CVS and/or AC)

Entered as:
0 + 1
2 + 1, etc.

Note:
- First row is total number of CVS.
- Second row is total number of AC.

14. Indication.
(: for antenatal diagnosis)

Entered as:
1 (= maternal and/or paternal age)
2 (= parental chromosomal rearrangement)
3 (= prior chromosomal anomaly)
4 (= X-linked disease (sexing))
5 (= metabolic disease)
6 (= haemoglobinopathy)
7 (= anxiety)
8 (= others),
followed by specification, e.g.
'5.Zellweger' or '2.Tri 18'.

Note:
- When in combination, the 'most important' indication taken first. (This is not always the one that carries the highest recurrence risk, e.g. 3+1: previous tri. 21 and mat. age 45, as such a patient would have come for antenatal diagnosis anyway regardless her age)
- Ad 8: chemo- and radiotherapy, diabetes mellitus, history of NTD etc. (often a 'wrong' indication for CVS).

15. Cong. Abn.
(: congenital abnormalities in family)

Entered as:
(compressed text) e.g. '3MRfm': 3 brothers of wife mentally retarded or 'NTD2ndfamp': 2 NTD's 2nd degree in family father or 'DSsp': trisomy 21 sister of father (all DS is tri.21 unless stated otherwise) or 'Ambl' (amblyopy), 'CHD' (cong. heart disease) etc.

Note:
This field is often a further specification of field no. 14, both rather roomy fields in order to accommodate e.g. prior chromosomal anomaly in a previous marriage or different congenital abnormalities in both families.

16. Gravidity

Entered as:
Total number of pregnancies (the present one included).

17. Parity.

Entered as:
Total number of pregnancies having ended ≥16 weeks gestational age (though see field no. 18).

18. Abortion.

Entered as:
Total number of pregnancies having ended <28 (!) completed weeks.

Note:
- So a notation like e.g. G9 P5 A6 is possible and means that of 8 previous pregnancies 3 ended in an abortion <16w., 3 ended between 16 and 28w., and 2 after 28w., while the 'Dutch' notation of G9 P5 A3 in this case would not have given an indication about possible 'immature' deliveries (≥16wk and <28wk), in which we are particularly interested in antenatal diagnosis.
- Further specifications in field no. 20 and no. 96.

19. AAP.
(= Abortus Arte Provocatus, induced termination of pregnancy)

Entered as:
Total number of induced terminations of pregnancy <28w.

Note:
- Regardless whether indication for AAP was psychosocial or medical ('therapeutic')
- Eventual specification in field no. 20, 96 or 15(e.g.ECHO-> ?chr.abn.->cordocent.->tri.18 (25w).

20. Prev. Problems.
(= problems in
- previous pregnancies
- previous deliveries
- in general history as far as possibly relevant for present pregnancy and eventual CVS or AC.

Entered as:
- Abbreviated text. Much used terms re. previous pregnancy/ delivery in alphabetical order:

Abr	(= Abruptio placentae)
APH	(= Antepartum Haemorrhage)
Br	(= Breech)
CS	(= Caesarean Section)
EP	(= Ectopic Pregnancy)
ENND	(= Early Neonatal Death)
FE	(= Forceps Extraction)
Hab	(= Habitual abortion)
ImmC	(= Immature Contractions <28w)
ImmD	(= Immature Delivery <28w)
IUFD	(= Intrauterine Fetal Death)
IUGR	(= Intrauterine Growthretardation)
Myom	(= Myoma/-ectomy in pregn.)
PIH	(= Pregnancy-induced hypertension)
PlPr	(= Placenta Problems like praevia, infarctions etc.)
PND	(= Post Natal Depression)
PPH	(= Post Partum Haemorrhage)
PremC	(= Premature Contractions ≥28 w.<37w.)
PremD	(= Premature Delivery -do-)
PROM	(= Premature Rupture Of Membranes)
Pr+IUD	(= Pregnant with IUD)
SB	(= Stillbirth)
SpAb	(= Sp.Abortion, only mentioned here if >1st trimester, otherwise in field no. 18)
TE	(= Thrombo-Embolic disease)

Thr (= Thrombosis only)
ThrAb (= Threatened Abortion, serious)
VE (= Vacuum Extraction)
etc.

- Often with time and length of duration in weeks if appropriate e.g. APH26wx3d.
- Overflow in field no. 96.

- Re. general history:
CARA (= CARA)
Card (= Cardiac disease)
Cot (= Cotdeath)
Diab (= Diabetes mellitus)
Epil (= Epilepsy)
Fert (= Fertility problems:
- AID = Artificial Insemination Donor
- Clom = Clomid induction
- Refrt = Refertilisation operation)
Hep (= Hepatitis)
Hyt (= Hypertension, pre-existent)
Oper (= Operation + specification)
Pulm (= Pulmonary disease + TB)
Psych (= Psychosocial disease)
Trfu (= Transfusion)
Thyr (= Thyroid disease)
UTI (= Urinary Tract Disease)
etc.

- overflow in field no. 96.

21. Bleeding.
(= vaginal bleeding between LMP and intake)

Entered as:
0 (= none)
1 (= old spotting)
2 (= old clots)
3 (= fresh spotting)
4 (= fresh clots)
5 (= others)

Note:
- In case more than one type present, most serious one taken (e.g. fresh clots and brownish for a few days afterwards).
- Time and duration in field no. 96.
- Ad 5: patient's description not fitting 1-4, e.g. 'pink', 'thinnish', 'watery', 'slimy', 'post coital like' (..!), 'reddish discharge', etc. Specification in field no. 96.

22. Discharge.
(= vaginal discharge between LMP and intake)

Entered as:
0 (= none)
+ (= present)

Note:
- Ad +: only when bothering patient either by quantity or complaints (say copious and itchy) to the extent as to suspect an infection and necessitating a cervical swab at intake.
- Specified in field no. 96.

23. Illness.
(: between LMP and intake)

Entered as:
0 (= none)
+ (= present)

Note:
- Type of illness specified in field no. 96 (often UTI etc. see field no. 24 for quick clue).

24. Medicines.
(= drugs taken between LMP and intake)

Entered as:
0 (= none)
1 (= analgesics)
2 (= anti-epileptics)
3 (= anti-histaminics/-emetics)
4 (= psycho-pharmaceutics)
5 (= a.biotics, sulpha, furadant.)
6 (= hormones)
7 (= vaginal suppositories)
8 (= others)
9 (= combination of drugs)

Note:
- Routine iron and vitamin preparations excluded.
- Hormonal treatment for induction also in field no. 20.
- Specifications in field no. 96.

25. Cigarettes.

Entered as:
Total number of cigarettes/day, or as '+' if yes, but quantity unknown

Note:
- '9' is 9 or more cigarettes/day.
- 'Stopped since pregnancy test positive' is entered as '0'.

26. Alcohol.

Entered as:
Total number of glasses/week, or as '+' if yes but quantity unknown

Note:
- '9' is 9 or more glasses per week (e.g. 1-2 beer daily).
- 'Stopped since pregnancy test positive' is entered as '0'.

27. Ultrasound.
(= date of ultrasound examination at intake)

Entered as:
yy - ww - mm

Note:
- This date is usually the same as date of intake (field no. 10). A later date however is entered in case of a follow-up US, this being the 'decisive' one (and result of 'intake US' specified in field no. 96)
- The total number of US before CVS is entered in field no. 74 (note data definition over there)
- In case 2nd US is even 'worse' than the intake-echo, intake-date is entered, also specified in no. 96.

28. Week.
(= gestational age at time of US, field no. 27)

Entered as:
ww + dd

Note:
As field no. 11.

29. CRL.
(= Crown Rump Length)

Entered as:
millimeters CRL + corresponding gestational age or as millimeters diameter gestational sac + corresponding gestational age.

Note:
e.g.(CRL): 34 = 10+1 or
(sac): d21 = 6+3. (d=diameter).

30. Uterus.
(= position of uterus)

Entered as:
A (= Anteverted)
R (= Retroverted)
M (= Midposition)
? (= unclear)

31. Placenta.
(= localization of placenta)

Entered as:
A (= Anterior)
P (= Posterior)
F (= Fundal)
I (= Inferior)
L (= Lateral, left or right)
S (= Surrounding)
+ (= 'seen', but not mentioned or not clear where exactly)
? (= not clearly visible)
– (= not seen, or seen but not mentioned).

Note:
Localization is that point of uterus over which thickest part of placenta is seen (and not place of cord insertion = field no. 34).

33. Yolksac.
(= localization of yolk sac)

Entered as:
field no. 32, except for 'S', becomes C (= (in the) Centre (of uterine cavity)).

34. Cord.
(= place of insertion umbilical cord)

Entered as:
field no. 32, except for 'S' (not possible)

35. Fetal Heart.

Entered as:
P (= Positive)
N (= Negative)
? (= doubtful)
– (in case field no. 40 = 0)

36. Abnormalities.

Entered as:
0 (= none)
1 (= fibroids)
2 (= 2nd sac)
3 (= multiple embryo's)
4 (= shadow = ?haematoma)
5 (= missed abortion)
6 (= blighted ovum)

7 (= negative discongruency)
8 (= positive discongruency)
9 (= other)

Note:
- Abnormalities outside uterus (i.a. cystic swellings not mentioned.
- A combination of up to 3 numbers can be entered.
- Because of considerable chance that initially diagnosed 'missed abortions' and 'blighted ova' later show up as viable (miscalculation etc.) and therefore actually should have been booked under '7', searching on either '5', '6' or '7' was always done with simultaneous reporting of the other two and corrected when necessary.
- Neg. and pos. discongruency ('7' and '8') stand for measurements of one week less resp. more than gestational age (and not ≤ and ≥).
- '9' is e.g. 'poor view', '?cong. abnormality like ut.bicornis, etc. (specified in field no. 96).

37. No CVS.
(= decision 'no CVS' taken at or after intake)

Entered as:
Field no. 12, and
0 (= CVS done)

or entered as:
US followed by a number from field no. 36 e.g.'US3' means 'at US gemelli' (and not AAP)

Note:
- As the number behind 'US' gives a further explanation of the ultrasound examination, a number in front of 'US' is a field no. 12 number e.g.'4US7' means 'no CVS because of abortionrisk and at US a negative discruency seen', which adds a different flavour compared with '4' only

Ad 8: = 'our advice'. Besides the reasons usually already evident at application or after consultation by phone and mentioned at field no. 12, it here also comprises e.g.
- suspect genital tract infection (awaiting C/S)
- too recent bleeding (<1w.)
- patient wants '100% risk-free', has a retroverted uterus with a posterior placenta and a history of infertility
- grossly overweight
- condylomata
- badly myomatous uterus.

PM: our advice based on US is entered as US followed by a number e.g. US2 (=2nd sac etc.), in which case the 2nd sac is 'in the way' and the sampling of proper villi not guaranteed (see also 'entered as' this field).

Ad 9: ='others' i.a. declining both AC and CVS, mostly because 'risk cong. abnormalities too small compared to risk of procedure', but also 'want to keep child anyway', 'would never submit to TOP', 'no insurance coverage', 'don't like it anymore' or 'just don't know' (these kind of descriptions are specified in field no. 96).

Extra Note:
- Our policy is: all patients with h/o NTD (besides their age indication) --> AC. regardless whether 1st, 2nd or 3rd

	degree and regardless of number of family members involved. From field no. 14 ('8'), no. 15 and this field = '0' one sees if a patient strongly desired otherwise and had CVS nevertheless.
38. 1st Session.[1] (= date of 1st CVS)	Entered as: yy - mm - dd
(48. 2nd Session, and so on)	Note: - If only probing and no aspiration done (cannula usually 'stuck' around internal os), then line nos 38 - 47 filled out, and field no. 37 = 'US' (further details in field no. 96) and field no. 75 = '0'. - The only patient with three sessions (as shown in field no. 75), had overflow in field no. 96).
39. Week. (= gestational age at CVS)	Entered as: Field no. 11.
40. CRL. (= Crown Rump Length)	Entered as Field no. 29.
41. Bleeding. (= vaginal bleeding between intake and CVS)	Entered as: Field no. 21.
42. Uterus. (= position of uterus)	Entered as: Field no. 31.
43. Placenta. (= position of placenta)	Entered as: Field no. 32.
44. Yolk sac. (= localization of yolk sac)	Entered as: Field no. 33.
45. Cord. (= place of insertion umbilical cord)	Entered as: Field no. 34.
46. Distance. (= length of cervical canal + distance from internal os to sampling site)	Entered as: mm + mm (= millimeters + millim.), or as mmT (= millimeters total). Note: e.g.'35+45', or '80T' (if level of internal os not clear), or '±80T' (if only approximately known).

[1] Nos 38 - 73: 'sampling block': consisting of 2 identical rows of fields in the screen lay-out in order to accommodate data from repeat sessions as well (row nos 38 - 47 is equel to nos 48 - 57, and row nos 58 - 65 = nos 66 - 73, see Figure 10.1).

47. Abnormalities.
(= Abnormalities before the actual procedure took off. Abnormalities arising during or after CVS are entered in field no. 64 and no. 65)

Entered as:
Field no. 36.

Note:
Again, all abnormaliteis are entered, otherwise comparison with abnormalities found at intake is not possible. E.g. a negative disproportion, even if of the same size as at intake, is entered as such (though strictly speaking not an 'abnormality', but if not entered it cannot be distinguished from 'intake mistake' and 'at sampling true to LMP'.

(row nos 48 - 57 = row nos 38 - 47, see footnote page 133)

58. Cannula.
(= cannula used)

Entered as:
P (= Portex™ cannula)
K (= King's College cannula)

Note:
PK (= Both cannula's used at CVS)

59. Insertion.
(= number of cannula insertions (actually aspirations) needed for sufficient quantity of villi)

Entered as:
Number, i.e. 1, 2, or 3.

Note:
And only sporadically 4.

60. Weight.
(= total (wet) weight of all villi as estimated at sampling, acc. to Simoni's weight chart, 1983)

Entered as:
Figure in milligrams.

Note:
Served also as feed-back when put against the definitive weight estimation in the laboratory.

61. Quality.
(= overall impression of villi's suitability for analysis)

Entered as:
G (= Good)
R (= Reasonable)
D (= Doubtful)
B (= Bad)

Note:
e.g. 20 mg good quality- and 25 poor peripheral villi is entered as weight '45' and quality 'G'.

62. Specimen.
(= contents of specimen)

Entered as:
0 (= no villi)
1 (= villi)
2 (= blood)
3 (= decidua)
4 (= membrane, ?chorion/amnion)
5 (= amniotic fluid)
6 (= mucus, brownish)

Note:
- Combination of several numbers is possible and a number between brackets means 'just a little bit' e.g. 1 (2) 4.
- Muscus is only mentioned if possibly from extra-embryonic space, mostly in conjunction with '4'. Clear, obviously cervical, mucus is not entered.

63. Success.
(= whether at CVS enough suitable villi present)

Entered as:
Y (= Yes)
N (= No)
D (= Doubtful)

Note:
- 'Y' entered here and no. 76 (Lab)= '0' means 'Laboratory failure'.
- 'N' entered here and no. 76 (Lab)= '0' means 'Sampling Failure'.

64. Problems.
(= problems during sampling)

Entered as:
0 (= no problems, easy sampling)
± (= medium, not all that easy)
+ (= clearly problematic), followed by text specification.

Note:
Much used specifications are
- bleed(ing), HbF + or -,
- 2nd sac (2nd sac obstructing)
- sten (stenosis cervical canal)
- oor (placenta out of reach)
- uncoop (patient uncooperative)
- fibr (fibroids in the way)
- bv (bad visualization)
- perp (cannula perpendicular to placenta)

Note:
'0' can be followed by some specific information like cx.pol. (= cervical polyp), bl.fill (= bladdermanipulation by instilling normal saline) etc.

65. Complications.
(= Complications after CVS)

Entered as:
0 (= no complications)
1 (= bleeding: much, frank)
2 (= spotting: little, brownish)
3 (= vaginal discharge, fluor)
4 (= fever)
5 (= pain, abdominal discomfort)
6 (= amniotic fluid leak)
7 (= other)

Note:
For time and duration of complaint the number is followed by
- CD = Complication Direct (<24H)
- CT = Complication Telephone (>24H <1 week)
- CL = Complication Late (>1wk<28w), followed by the number of days, weeks etc.

e.g.: 1 CDTx3d(US-) means:
Bleeding at sampling and (as told when reporting result by phone) lasting for 3 days, while ultrasound check directly following CVS was normal (i.e. not showing haematoma or something).

(CL ≥28w: see field no. 93)

(row nos 66 - 73 = row nos 58 - 65, see footnote page 133)

74. no. of US.
(= total number of ultrasound examinations done before CVS)

Entered as:
number

Note:
- Only US done at AD-OPD are counted as well as
- US elsewhere in case it was done on our request (after e.g. an ambiguous intake-US at ours) and when followed by CVS.

- Not counted are the
· Us made elsewhere (before referral for antenatal diagnosis)
· US done at sampling that cancelled CVS (e.g. because FH negative)
· US made in e.g. 14wk. as follow-up when after intake AC was preferred.
· US made at AZG in another Dept. (e.g. 'Endocrinology' after induction, before referral AD)

So, the number eventually entered represent an as true as possible picture of the number of US examinations (extra) needed in association with CVS.

75. No. of Sessions.
(= Number of CVS sessions)

Entered as:
number

Note:
- Twin = 1 session
- More than one session: see specification in field no. 96.

76. CVS result.[1]
(= Laboratory result of CVS)

Entered fully,
e.g. '46,XY' or
'46,XX, no Sanfilippo' or
'46,XY(70%)/92,XXYY(30%).

Note:
- Actually a '+' (abnormal) or '–' (normal) was entered first followed by karyotype for ease of searching.
- Inversions and balanced translocations also preceded by a '+' and later 'corrected' by hand, this worked far easier than having to find them when they are booked as normal.

77. Sex.
(= whether sex of infant was made known to parents)

Entered as:
K (= Known)
N (= Not known)
H (= 'Half': one knows, the other does not)[2]
0 (= not relevant).

Note:
- Ad '0': As this category 'sex' was created to have an idea about how many wanted to know the sex of their baby

[1] Nos 76 - 86: 'after CVS and before 28 week block', containing data about laboratory results and complications before 28 week, including details about fetal loss, first trimester abortions included.
[2] It was always one way (which?)

without having to, all indications 'X-linked' (field no. 14) and results like XXY, XXX etc. are entered as '0' and not as 'K'.
- This is at the time parents were informed about the result of the test, i.e. 1 week after CVS and 3 weeks after amniocentesis. If they changed their mind later and were informed (e.g. by their G.P.) about the sex after all, is only partly known to us (only in case our own Dept. was the referrer) and is not entered.

78. Cervix.
(= Laboratory result of cervical smear taken before each sampling)

Entered as:
0 (= 'sterile' or 'no pathogenic organisms detected'
1 (= Candida)
2 (= Trichomonas)
3 (= Staphylococci)
4 (= E.Coli)
5 (= Streptococci)
6 (= Enterococci)
7 (= Gardnerella)
8 (= 'Anaerobes')
+ (= 'Micro-organisms seen but no growth')

Note:
- Combination of numbers is possible (e.g. '17').
- In case of 2 sessions CVS, result is entered as e.g. '0+4'.
- Cx. swab only sporadically taken at intake and mentioned not here but in field no. 96.
- Ad 5: = all beta-haemolytic streptococci.

79. MSAFP b. & a.
(= Maternal serum alpha-fetoprotein before and after CVS)

Entered as:
Figure in ng/ml.

Note:
e.g. as 12/12 (decimals rounded), or as 12/-, in case 2nd specimen not taken ('forgotten', 'lost' etc.).

80. MSAFP 16w.
(= Maternal serum alpha-fetoprotein at around 16th week or later; the exact week entered in field no. 81)

Entered as:
Field no. 79.

Note:
- In case CVS is followed by AC (e.g. for metabolic) then amniotic fluid AFP entered as e.g. '52 A'.
- See field no. 96 for further details of abnormal results like follow-up etc.

81. AFP week.
(= gestational age in weeks when MSAFP was taken)

Entered as:
number (16, 17 etc.)

Note:
16 + 0 = entered as '17' (= 17th week).

82. Fetal Loss.
(= all 'fetal loss' <28w.)

Entered as:
0 (= no fetal loss)
SpAb (= Spontaneous Abortion)
ThAb (= Therapeutic Abortion, i.e. induced)
AAP (= Abortus Arte Provocatus for psychosocial reasons)

Note:
- So, all immature deliveries are entered, whether surviving or not, + and later corrected according to the kind of 'abortion definition' one prefers.
- Details of fetal loss are put in field no. 86 ('blighted', result histopathology etc.)

83. F.L. Week.
(= week of fetal loss)

Entered as:
Number, see field no. 81.

84. Amniocentesis.
(= date of amniocentesis)

Entered as:
yy - mm - dd

85. AC result.
(= Laboratory result of amniocentesis)

Entered fully, like no. 76.

86. Details.
(= Details of fetal loss post-amniocentesis, <28w.)

Entered as text.

Note:
- Like result histopathology, infection, amniotic fluid leak etc. with overflow in field no. 96.
- Problems ≥28wk. are found in no. 93.

87. Delivery.[1]
(= date of all deliveries ≥28w)

Entered as:
yy + mm - dd

Note:
= '0' if e.g. field No. 82 is filled.

88. Week.
(= week of delivery menstrual age or based on US in case of unreliable LMP)

Entered as:
ww + dd

Note:
'Term' is entered in case neither LMP nor US is known (e.g. in the drop-out group), while our follow-up reported 'full term'.

89. Mode.
(= mode of delivery)

Entered as:

VD sp	(= Vertex spontaneous)
VD in	(= Vertex induced)
BR as	(= Breech assisted)
BR ex	(= Breech extracted)
VE	(= Vacuum Extraction)
FE	(= Forceps Extraction)
CS el	(= C.Section elective)
CS em	(= C.Section emergency)

Note:
- Details in field No. 93 ('fetal distress', 'no progress', 'previous scar' etc. Overflow in field no. 96 in case e.g. induction + failed vacuum + emergency caesar.
- All VE and FE are considered emergency.

90. Weight.
(= weight of infant)

Entered as:
Figure in grammes.

[1] Nos 87 - 94: 'delivery block', containing data about pregnancy- and delivery(problems) ≥28 week.

91. Abn. Infant. (= abnormalities infant at birth)	Entered as 0 (= no abnormalities) + (= abnormalities present) Note: - Ad '+': not only congenital abnormalities, but also 'small-for-dates', 'prematurity' etc. - SFD = <10 percentile (acc. to Kloosterman) and prematurity = ⩾28 wk. <37 wk. - Specification in field No. 95.
92. Abn. Placenta. (= abnormalities placenta)	Entered as: 0 (= no abnormalities) + (= abnormal) P (= histopathology done) Note: - Ad 'P': a special category comprising 'normal' placentae recovered for morphological study. - Details in field no. 95 and no. 96
93. P/D Problems. (= Problems in pregnancy and at delivery. If resulting in fetal loss: data entered in field no. 86)	Entered as text. Note: - Usually ⩾28w, but also e.g. a passing APH in 26w. x 3/52 - Abbreviations as set out in field no. 20. - Overflow in field no. 95.
94. Place. (= place of delivery)	Entered as: 1 (= home) 2 (= 2nd. referral to hospital) 3 (= hospital) A (= AZG, our department) – = not known) Note: - Ad '3': also if done by G.P. or midwife. - Ad 'A': a secondary referral to our Dept. remains 'A' instead of '2', in order to have easy access to a group of patients with 'complete' obstetrical records, and 'corrected' in searches on '2' when necessary.
95. Child. (= details of child and placenta)	Entered as text. Note: - Like percentiles, true knot, exchange transfusion etc. - '0' = no child (e.g. after sp.ab.)
96. Notes. (= 256 character textfield for further details and as overflow for data not fitting in their respective fields)	Entered as free text Note: - Also including specifications such as 'for motility study', 'villi for SCEM', 'interview socio-girls' and lots of telling quotations.

List of frequently used abbreviations not always explained in the right hand column 'notes' category.

AAP	Abortus Arte Provocatus (psychosoc)
AC	Amniocentesis
AFP	Alpha-fetoprotein
AZG	Academisch Ziekenhuis Groningen
Chr	Chromosomal
Cong	Congenital
C/S	Culture and Sensitivity
CVS	Chorionic Villus Sampling
Cx	Cervix
DS	Down's syndrome
ECHO	Echoscopy
FH	Fetal Heart
GP	General Practitioner
HbF	Fetal Haemoglobin
IU(C)D	Intrauterine (Contraceptive) Device
LMP	Last Monthly Period
NTD	Neural Tube Defect
OPD	Out Patient Department
SFD	Small-for-date
Tri	Trisomy
TOP	Termination of Pregnancy
US	Ultrasound

11 APPENDIX

DEEL I AANMELDING

gegevens naamplaatje

+ polisnummer

zie intake: vs. 23

- verwezen door: 1. huisarts, 2. vroedvrouw, 3. gynaecoloog, (4) eigen kliniek, 5. zelf*. Visser.
- eerste dag van de laatste menstruatie: 13.10.85 (datum)
- rhesus factor: (1) positief, 2. negatief.
- indicatie:
  1. leeftijd
  2. translocatie bij man/vrouw, t.w.
  3. chromosoomafwijking in voorgeschiedenis, t.w.
  (4) x-gebonden aandoening, t.w. hydrocephalie zr
  5. stofwisselingsstoornis, t.w.
  6. haemoglobinopathie, t.w.
  7. emotioneel
  8. anders

A/C

in dit kader niet in computer, maar wél invullen.

*desbetreffende getallen omcirkelen.

DEEL II INTAKE-gesprek en ECHO-onderzoek

gegevens naamplaatje

+ polisnummer

zie intake: No. 23.

INTAKE

- vindt plaats op 9.12.85 (datum),
- bij zwangerschapsduur van: 1. 12 wk, 1 dg, 2. onzeker.
- vindt niet plaats vanwege: 1. intercurrente abortus, 2. dreigende miskraam, 3. heeft zich bedacht. reden:
- anamnese: 1. gravida: 5 2. para 2 3. sp. abortus (≤ 22 wk) 2 4. AAP....
- complicaties eerdere zwangerschap/bevalling:
  1. nee, (2) ja — t.w.: 2x hydrocephalus. NAG. zie cervix (2x NVZV.)
- bloedverlies:
  (1) nee, 2. ja — oud / vers, hoeveelheid:
- afscheiding:
  1. normaal, 2. teveel — riekend: ja / nee — kweek:
- cigaretten:
  1. nee, 2. ja — aantal per dag:
- alcohol:
  1. nee, 2. ja — glazen/wk:
- medicijnen: Stollingsstoornis ontkent.
  1. nee, 2. ja — t.w.: hoeveelheid:
- drugs:
  1. nee, 2. ja — t.w.: hoeveelheid:
- *lichamelijk onderzoek:
  (1) nee, 2. ja — bevindingen:

ECHO

- vindt plaats op: 9.12.85 (datum)
- bevindingen conform zwangerschapsduur:
  (1) ja 2. nee — zie echoverslag
- datum CVB: (1) ja, 2. nee — reden:
- herhalingsecho('s):
  1. nee, 2. ja — zie verslagen

CRL = 0.9 = 7 wk. uterus norm. doorlies. n.z. haas. insertie voor? plac. randem. A/C ok niet. ok.

niet in computer wél invullen

*alleen op indicatie

DEEL III CHORIONBIOPSIEVERSLAG

gegevens naamplaatje

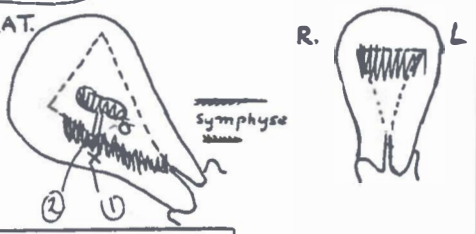
+ polisnummer

gynaecoloog: HANTINGH
geneticus : BREED
echo : BOOGERT
tijd : 11.30

- datum: 23.12.85
- bij zwangerschapsduur: 10 wk, 0 dg.
- bloedverlies: (1) nee, 2. ja oud / vers, hoeveelheid: ....
- uterus: (1). AVF, 2. RVF, 3. strekstand, 4. onduidelijk.
- CRL 28 mm. overeenkomend met graviditeitsduur: 9 wk, 4 dg.
- coractie: (1) positief, 2. negatief.
- placentalocalisatie: 1. ant., (2) post., 3. in fundo, 4. onder, 5. lateraal, 6. andere: ....
- structuur placenta: (1) normaal, 2. abnormaal, t.w.:
- procedure: (1). vlot, 2. moeizaam
- instrument: (1) portex cannula, 2. K.C. (silver)cannula, 3. borstel, 4. ander:
- route: (1) transcervicaal, 2. ~~transabdominaal~~
- cervixkweek: (1) ja, 2. nee. (MSaFP voor/na) Kleihauer-Betke ja/nee.

Beschrijving van procedure:

- vulva chloorhexidine,
- vagina + portio: betadine jodium.
- lengte cervicaal kanaal: 4 cm
- lengte ostium internum tot plaats biopsie 4 cm.
- spuit: 30 cc., vacuum: 15 cc.
- placenta arceren; navelstreng, dooierzak en vrucht intekenen; biopsieplaats aangeven (x en nr.)



- 1e biopsie: (1) villi, (2) + bloed, (3) + decidua, 4. + vlies 5. + vruchtwater, (6) + mucus bruin/helder. villi 6 mg
- 2e biopsie: (1) villi, (2) + bloed, 3. + decidua, 4. + vlies, 5. + vruchtwater, 6. + mucus bruin/helder. villi 42 mg
- 3e biopsie: 1. villi, 2. + bloed, 3. + decidua, 4. + vlies, 5. + vruchtwater, 6. + mucus bruin/helder. villi .. mg
- totaal gewicht villi: 48 mg.
- kwaliteit villi: (1). goed, 2. matig, 3. slecht.
- complicaties na ingreep: (1) nee, 2. ja. coractie: ... , haematoma: ... vloeien: ... , anderszins: ....
- anti D: (1) nee, 2. ja.
- haemoglobine onderzoek: (1) nee, 2. ja. maternaal: .. , foetaal: ..
- onderzoek: (1) karyotypering, 2. ander, t.w.: ...
- CVB niet verricht, reden:...

AZG

niet in computer, wél invullen.

DEEL IV RESULTAAT en BELOOP ZWANGERSCHAP

gegevens naamplaatje

+ polisnummer

- biopsie gelukt: (1.) ja, 2. nee, reden:
- analyse gelukt: (1.) ja, karyotype: 46xx
  2. nee, reden:
- cervixkweek: (1.) neg, 2. pos., te weten:

uitslag doorgebeld, d.d. 27-12-85 ; aan echtpaar/huisarts,
weet geslacht: (ja) / nee.

- complicaties op dag van bellen:
  (1.) nee, 2. bloedverlies, 3. vruchtwaterverlies, 4. buikpijn/kramp,
  5. afscheiding, 6. koorts, 7. andere, te weten:
- AAP: 1. nee, 2. ja, waar: afwijking geconfirmeerd?
- latere abortus: 1.nee, 2. ja, week: en symptomen:
  PA na-onderzoek: nee/ja:
  karyotypering: nee/ja
- (echo) follow-up ≠ 16 wk conform: 1. ja, 2. nee. zie echoverslag.
- (echo) follow-up ≠ 32 wk conform: 1. ja, 2. nee. zie echoverslag.
- beloop zwangerschap: (1) ongestoord, 2 gestoord, t.w.:
- partus datum: 11-7-86
- graviditeitsduur: 38 wk, 3 dg.
- geslacht: 1. jongen, (2.) meisje
- geboortegewicht: 2410 G gr.
- lengte: 46 cm 5
- gezond: (1.) ja, 2. nee, t.w.
- placentagewicht: gr.
- placenta normaal: 1. ja, 2. nee, t.w.: P.A.:

voetlengte = 7.5
borstomv = 30
buikomv = 29
... = 8.5
hoofdomtr = 32

→ AZG

AZG

niet in computer,
wél invullen

... 2/2/86

11.2 Table of indications for antenatal diagnosis in the Netherlands (as from 1985, in Dutch)

De indicaties voor prenataal chromosomenonderzoek zijn de volgende:

- zwangeren van 36 jaar en ouder i.v.m. een verhoogde kans op een kind met een chromosoomafwijking (o.a. trisomie 21, 18, 13 of 47, XXY)*;
- zwangeren wier echtgenoot 55 jaar of ouder is, eveneens i.v.m. een verhoogde kans op een kind met een chromosoomafwijking*;
- echtparen die reeds eerder een kind met een chromosoomafwijking ter wereld hebben gebracht; gebleken is dat de herhalingskans in deze situatie verhoogd is vergeleken met het risico in de normale populatie;
- echtparen waarvan één der partners drager is van een chromosoomtranslocatie; het risico van een kind met een ongebalanceerde chromosoomafwijking is in deze situatie verhoogd. De grootte van het risico is afhankelijk van het type translocatie en de vraag of de man dan wel de vrouw drager is.
- zwangeren bij wie d.m.v. ultrageluidonderzoek aanwijzingen zijn gevonden voor een foetale misvorming; vastgesteld is dat in deze gevallen de kans op een foetale chromosoomafwijking sterk verhoogd is;
- zwangeren die draagster zijn van een X-gebonden erfelijke ziekte (bijv. hemofilie, progressieve spierdystrofie van Duchenne, X-gebonden mentale retardatie), welke niet door biochemisch onderzoek van vruchtwatercellen is vast te stellen; in deze situatie zal chromosoomonderzoek kunnen aantonen, of de vrucht van het vrouwelijk geslacht is, waarbij de ouders hun zwangerschap gerustgesteld kunnen vervolgen, omdat een meisje niet aan de betreffende erfelijke ziekte zal lijden; bij een mannelijke vrucht is de kans dat deze de ziekte wel zal hebben echter 50% en kunnen de ouders op grond van dit hoge risico eventueel besluiten hun zwangerschap af te breken;
- zwangeren die zelf een neuraalbuisdefect hebben of eerder een kind met een open neuraalbuisdefect ter wereld hebben gebracht en bij wie vruchtwater wordt onderzocht op het alpha-foetoproteïnegehalte; van het voor dit laatste doel afgenomen vruchtwater kunnen ook de foetale cellen worden gekweekt en kan chromosoomonderzoek worden verricht om de ouders de teleurstelling te besparen, dat er na een evt. geruststelling m.b.t. een open neuraalbuisdefect- toch een ernstig gehandicapt kind met een chromosoomafwijking wordt geboren;
- zwangeren die eerder een kind met een erfelijke stofwisselingsziekte ter wereld hebben gebracht en bij wie vruchtwater wordt afgenomen voor (micro)biochemische analyse van de vruchtwatercellen; van een deel van deze cellen wordt chromosoomonderzoek uitgevoerd om dezelfde reden als hierboven vermeld;
- zwangeren die eerder een kind met een aangeboren misvorming ter wereld hebben gebracht en bij wie de kans op een chromosoomafwijking verhoogd is;

Indicaties voor een alpha-foetoproteïne onderzoek zijn:

- echtparen waarvan één der partners zelf een neuraalbuisdefect heeft;
- echtparen die reeds een kind met een neuraalbuisdefect ter wereld hebben gebracht;
- echtparen waarbij er één eerstegraads bloedverwant, twee tweedegraads bloedverwant of drie of meer derdegraads bloedverwanten met een open neuraalbuisdefect voorkomen;
- zwangeren die geneesmiddelen gebruiken, waarvan bekend is of het vermoeden bestaat dat deze een open neuraalbuisdefect kunnen veroorzaken;
- alpha-foetoproteine onderzoek kan daarnaast ook worden verricht op vruchtwatermonsters die voor andere doeleinden dan een verhoogde kans op een neuraalbuisdefect zijn afgenomen; voorbeelden zijn vruchtwatermonsters welke voor prenataal chromosoomonderzoek of (micro)biochemische analyse van erfelijke(stofwisselings)ziekten worden afgenomen. De alpha-foetoproteïne-bepaling is relatief eenvoudig en voorkomen wordt, dat echtparen die gerustgesteld worden m.b.t. een foetale chromosoomafwijking of een erfelijke ziekte, onverwacht geconfronteerd worden met een ernstig gehandicapt kind met een open neuraalbuisdefect. In dergelijke gevallen kan geen apart tarief voor het alpha-foetoproteïne-onderzoek in rekening worden gebracht.
- verhoogd serum αFP;

*t.t.v. punctie (geldt voor AP en CVB!)

KWALITEITSNORMEN AMNIOCENTESE & CHORIONVILLI BIOPSIE*

RICHTLIJNEN VOOR GYNAECOLOGEN WERKZAAM IN HET ANTENATALE DIAGNOSTIEK TEAM:

- De gynaecologen dienen de ingrepen geleerd te hebben en intensieve contacten te blijven onderhouden met een (inter-)nationaal erkend centrum voor Antenatale Diagnostiek.
- Alvorens tot chorionvilli biopsie op indicatie over te gaan dient men de techniek te leren bij minstens 50 abortus arte provocatus-patiënten.
- De eerste 15 chorionvilli biopsieën op indicatie dienen succesvol onder supervisie van een ervarene, te worden verricht.
- De eerste 25 tweede trimester-amniocentesen dienen onder supervisie te worden verricht.
- De gynaecoloog moet per jaar tenminste 50 chorionvilli biopsieën en/of amniocenteses verrichten (dit getal geldt per operateur, niet per centrum). Om de continuiteit te waarborgen dient elk centrum over minstens 2 operateurs te beschikken.
  Binnen 2 jaar na opstarten van een nieuw centrum moet een aantal van 100 verrichtingen per jaar plaatsvinden.
- Aangezien tot op heden de echoscopische localisatie (dit geldt met name voor de chorionvilli biopsie) als zeer belangrijk wordt ervaren dient de operateur voldoende beheersing te hebben van de echoscopische techniek voor de diagnostiek van jonge zwangerschappen.
  Goed teamwerk tussen echoscopist en operateur is een vereiste.
- Het team dient de resultaten, complicaties en follow-up gegevens te rapporteren in de vorm van een jaarverslag.
  Rapportage geschiedt aan het Klinisch Genetisch Centrum waarmee men samenwerkt.
- In het centrum moet de mogelijkheid c.q. de bereidheid aanwezig zijn om in voorkomende gevallen de zwangerschap af te breken.
- Voorts moet hij of zij in staat zijn tot het geven van eenvoudige erfelijkheidsadviezen, volgens de ministeriële aanwijzingen, in nauwe samenwerking met een Klinisch Genetisch Centrum.

1. Voor wat betreft chorionvilli biopsie kan in het algemeen gesteld worden dat het gaat om een nieuwe techniek ("toegepaste research procedure") waarvan nóch de procedure zelf, nóch veiligheid en effectiviteit van de diverse gebruikte methoden tot op heden voldoende geëvalueerd zijn. Resultaten van onderzoeken die momenteel in binnen- en buitenland gaande zijn, zullen in de naaste toekomst meer duidelijkheid moeten verschaffen
2. In dit voorstel worden de procedures van 2e trimester amniocenteses en chorionvilli biopsie bewust niet nader omschreven vanwege verschillen in technische uitvoering. Uitgangspunt is dat men de betreffende techniek leert in een centrum met goede resultaten

EISEN TE STELLEN AAN HET ZIEKENHUIS:

- Er moet een contract zijn met een stichting die de bevoegdheid heeft verkregen tot het verrichten van cytogenetisch onderzoek.
- Aan het gynaecologisch/echografisch team dient realtime echo-apparatuur met hoog oplossend vermogen ter beschikking te staan
- De ingreep moet op een vaste dag, tenminste 1x per week, kunnen worden verricht in een behandelkamer waar geen septische patienten worden onderzocht of behandeld.
- Voor beoordeling van materiaal verkregen bij chorionvilli biopsie moet geschikte microscopische apparatuur (dissectie microscoop of omkeer microscoop) beschikbaar zijn.
- Het ziekenhuis dient een vergunning te hebben voor abortus arte provocatus.

EISEN TE STELLEN AAN DE PROCEDURE:

- De ingreep wordt voorafgegaan door het intake gesprek, waarin mondelinge en eventueel schriftelijke en/of audiovisuele informatie wordt gegeven.
- Het intake gesprek vindt bij voorkeur plaats op een andere dag dan de ingreep zelf en slechts bij uitzondering in een ander ziekenhuis dan waar de ingreep plaatsvindt.
- Tijdens het eerste bezoek wordt echografisch onderzoek verricht, onder meer om de vitaliteit van de vrucht vast te stellen en om te kunnen anticiperen op eventuele technische problemen (bijvoorbeeld in geval van myomen of ongunstige placentalocalisatie).
- bij chorionvilli biopsie zijn aanwezig behalve de operateur, een verpleegkundige en een ervaren echoscopist. Een in de cytogenetica ervaren klinisch geneticus of een cytogenetisch analist moet beschikbaar zijn.
- Ten alle tijde dient men bij het afbreken van de zwangerschap op genetische indicatie de eerder gestelde diagnose te verifiëren (chromosomenonderzoek, pathologisch-anatomisch eventueel biochemisch en bacteriologisch onderzoek).

DE CHORIONBIOPSIE (VLOKKENTEST)

Werkwijze

De bedoeling is, om met een dun slangetje (doorsnede 1½ mm), dat via de schede in de baarmoeder is gebracht, een klein stukje van de moederkoek op te zuigen. Dit gebeurt onder gelijktijdige echoscopische kontrole, terwijl u op de onderzoekstafel ligt, met de benen in de steunen, zoals bij het algemeen gynaecologisch onderzoek. U zult er nauwelijks iets van voelen, verdoving is dan ook niet nodig.

Voordeel

Het voordeel is, dat de test vroeg in de zwangerschap kan worden verricht (bij ± 9 weken) en dat de uitslag snel bekend is (± 1 week).
Als u op grond van de uitslag de zwangerschap wilt laten afbreken, dan kan dat nog op tamelijk eenvoudige wijze poliklinisch gebeuren (d.m.v. zuigcurettage).

Nadeel

Een nadeel is het risiko van een miskraam door de ingreep. Hoe groot is (nog) niet precies bekend, waarschijnlijk iets groter dan bij de vruchtwaterprik. Evenmin weten we of er in het verdere verloop van de zwangerschap ook meer kans is op problemen als voortijdig vruchtwaterverlies, e.d.

Beperking

Een beperking van het onderzoek is, dat we voorlopig alleen afwijkingen van het erfelijkheidsmateriaal (de chromosomen) kunnen opsporen, zoals b.v. mongoloïdie. Voor aandoeningen als "open ruggetje" e.d., zijn we op het onderzoek van bloed of vruchtwater in de 16e week aangewezen. Dat geldt ook voor de meeste stofwisselingsziekten.

Mislukken

Tenslotte, kan de test mislukken? Ja, ofwel omdat we de juiste plek om weefsel af te nemen niet goed kunnen bereiken (doordat b.v. de baarmoeder te sterk gekanteld is), ofwel omdat in het laboratorium de chromosomen onvoldoende zichtbaar gemaakt kunnen worden (dat kan b.v. gebeuren als het weefselstukje onvoldoende zich delende cellen bevat).
We zijn dan alsnog op een vruchtwaterprik in de 16e week van de zwangerschap aangewezen.

A. Mantingh.

P.S.: Kunt u op de dag van de vlokkentest met volle blaas komen?

HET VRUCHTWATERONDERZOEK

Om welke afwijkingen gaat het?

Bij het algemene vruchtwateronderzoek gaat het meestal om 2 groepen aangeboren afwijkingen. De ene groep betreft de "neuraalbuisdefecten". Dit zijn aandoeningen als 'open ruggetje', 'open hoofdje', e.d. Het is echter *alleen* mogelijk de *open* defecten op te sporen. De gesloten afwijkingen blijven onopgemerkt. Daartoe behoort het waterhoofd, maar ook sommige -meestal minder ernstige- vormen van open ruggetje. Voor deze groep geeft het vruchtwateronderzoek dus *geen* 100% zekerheid. Soms is nader echoscopisch onderzoek nodig.

De andere groep betreft aangeboren afwijkingen die het gevolg zijn van veranderingen in het erfelijkheidsmateriaal (de chromosomen). Daarvan is "mongoloidie" de belangrijkste, maar er zijn ook andere, die minder vaak voorkomen. Het laboratorium komt *alle* zichtbare chromosoomafwijkingen op het spoor en het onderzoek is voor deze groep dus *wel* 100%. Ook zien we of het een jongetje of een meisje zal worden. Stelt u er geen prijs op dit ook te weten, dan houden we deze kennis voor ons.

P.M. Er kunnen veel meer afwijkingen door vruchtwateronderzoek bepaald worden, maar daarvoor geldt meestal dat we van te voren moeten weten om welke aandoening het precies gaat. Dus als u een verhoogd risico hebt omdat een bepaalde afwijking in de familie voorkomt (bepaalde stofwisselingsstoornissen bijvoorbeeld), kunt u ook in aanmerking komen voor antenatale diagnostiek. Maar dat voert ons nu te ver.

Eerste gesprek en echo-onderzoek (maandagmiddag, polikliniek)

Voordat u een gesprek met de arts hebt, wordt eerst een ultrageluidsonderzoek van de baarmoeder gedaan. We kunnen dan nagaan of de zwangerschap niet minder ver of juist verder is dan we op grond van de laatste menstruatie hebben berekend. Dit is van belang omdat de vruchtwaterprik niet eerder dan de 16e week gedaan kan worden, omdat er dan pas voldoende vruchtwater is. Tijdens het gesprek wordt dan de precieze datum afgesproken (in elk geval altijd op een woensdagochtend, dan staat het laboratorium ervoor klaar). Verder is het gesprek bedoeld voor nadere uitleg over de verdere gang van zaken, het prikken zelf, complicaties (zie onder), vragen van uw kant, enz.

Vruchtwaterpunctie (woensdagochtend, 1e verdieping)

U meldt zich weer aan de balie op de polikliniek. Daarna gaat u naar de eerste verdieping, de pijlen "vruchtwateronderzoek" volgend, richting wachtkamer. We roepen u als het zover is. Het prikken zelf is gauw uitgelegd. Eerst wat bloed uit de arm, vervolgens weer een echo (bij lege blaas, dus u hoeft thuis geen urine te sparen) om een goed plekje uit te zoeken. Daarna onderbuik desinfecteren, plaatselijke verdoving met een klein dun naaldje en dan de vruchtwaterprik met een langere naald, ergens tussen navel en schaambeen. We zuigen ± 20 cc. vruchtwater op, ongeveer 1/8 van de totale hoeveelheid, die vlot weer aangemaakt wordt. Als u Rhesus negatief bent, krijgt u na afloop een injectie anti-D ter bescherming. Wanneer in de loop van de middag de plaatselijke verdoving uitgewerkt raakt, kunt u nog wel wat hinder hebben van een trekkerig gevoel onder in de buik. Soms houdt u er een paar dagen een kleine blauwe plek aan over, maar al met al hoeft u zich die dag niet speciaal te ontzien.

Wat zijn de risico's?

Het belangrijkste is het risico op een miskraam. Dat kan komen door een heftige kramptoestand van de baarmoeder als reactie op de prik, door een infectie, of door veel vruchtwaterverlies, maar meestal 'zomaar' zonder duidelijke oorzaak. Dit risico is niet groot (kleiner dan 1%), maar reëel genoeg om u er bij uw besluit vruchtwateronderzoek te laten doen, van bewust te zijn. De kans op aanprikken van de vrucht is uitermate klein.

De uitslag

Deze is voor de "neuraalbuisdefecten" binnen een paar dagen bekend en wordt u alleen doorgebeld als u hiervoor een verhoogd risico had. Voor de "chromoscoomafwijkingen" is de uitslag echter pas na ongeveer 3 weken bekend, omdat de vruchtwatercellen zich eerst goed moeten vermenigvuldigen voordat er genoeg zijn om onderzoek op te kunnen doen. Bij hoge uitzondering komt het wel eens voor dat de "kweek niet aanslaat" en er te weinig cellen zijn om een definitieve uitspraak te doen. Met de "kwaliteit" van de vrucht heeft dat niets te maken, maar de kwestie is dan wel: overprikken of niet.
Wij bellen de uitslag zelf, aan u persoonlijk door, zodra wij hem van het laboratorium hebben gekregen (meestal dezelfde dag nog), behalve als deze niet goed is. In dat geval stellen we uw huisarts op de hoogte om met u te kunnen overleggen wat er verder moet gebeuren.

G.H.A. Visser, A. Mantingh, vrouwenartsen.
secretariaat: 050-613080

STICHTING VOOR ERFELIJKHEIDSVOORLICHTING

De Stichting voor Erfelijkheidsvoorlichting heeft ten doel de bevordering van erfelijkheidsvoorlichting en alle daarvoor nodige diagnostische onderzoekingen, behandelingen en adviezen in het bijzonder ten behoeve van de bevolking in de regio Noord-Oost Nederland. De Stichting is gevestigd te Groningen.

A. Deusinglaan 4
9713 AW Groningen
Tel.: 050-117001

Aan:
- Gynaecologen en
- Inspekteurs der volksgezondheid in Groningen, Friesland, Drenthe en Overijssel

Betreft: chorionbiopsie

Ref. nr: ..GA/IB/84/042..... GRONINGEN, ...31.oktober..... 19.84.

Geachte collega,

Tot nu toe werd aan zwangeren die in aanmerking komen voor antenatale diagnostiek de mogelijkheid geboden een amnionpunktie in de 16e week van de graviditeit te ondergaan.
Sinds 2 jaar zijn in diverse centra nieuwe methoden ontwikkeld met als doel deze vorm van antenatale diagnostiek in een deel der gevallen te vervangen door de "vlokkentest" in de 8e-9e week van de zwangerschap.
Vanaf 1 november 1984 zal deze test ook in het Academisch Ziekenhuis Groningen op beperkte schaal kunnen worden uitgevoerd.
Zoals u wellicht weet biedt deze test het grote voordeel van een diagnose in een vroeg stadium van de zwangerschap en bovendien een uitslag binnen enkele dagen na afname van het materiaal (bij vruchtwaterpunktie bedraagt deze termijn 15 tot 20 dagen).

Indicaties.
Bedacht dient te worden, dat vooralsnog alleen chromosomale diagnostiek kan worden gedaan. Zwangeren met b.v. een verhoogd risico op een kind met een neuraalbuisdefekt blijven op het vruchtwateronderzoek voor AFP-bepaling aangewezen. Voor enzymdiagnostiek hangt het af van het type stofwisselingsziekte of enzymaktiviteit in vruchtwater of chorionvillibiopsie materiaal kan worden bepaald.

Kwantitatief de belangrijkste indikatie voor antenatale diagnostiek vormt de leeftijd van de zwangere. Officieel ligt de leeftijdsgrens nog steeds bij 38 jaar ten tijde van de uitgerekende datum.

Komplikaties.
Ten aanzien van het risico op een abortus ten gevolge van de ingreep dient opgemerkt te worden dat dit feitelijk nog niet bekend is; ook internationaal is de test nog op onvoldoende ruime schaal toegepast om een betrouwbaar abortuspercentage te kunnen geven.
Het risico op een abortus van een vruchtwaterpunktie bedraagt ± 0,5%.
Er zijn aanwijzingen dat het risico van de chorionvillibiopsie in dezelfde orde van grootte ligt (laagste schatting 0,5% - hoogste schatting 4%). Over de kans op problemen later in de zwangerschap (groeivertraging ?), of het risico van vruchtbeschadiging is nauwelijks iets bekend.

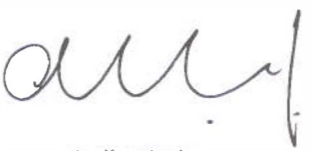
Uitvoering.
De aanvraagprocedure (machtiging door de ziekenfondsen) verschilt niet van die van het vruchtwateronderzoek.
De praktische gang van zaken is dezelfde als voor het vruchtwateronderzoek. Dat wil zeggen aanmelding bij mw. I. Koop, telefoon: 050-613080, voor bezoek aan de polikliniek antenatale diagnostiek - een samenwerkingsverband tussen de afdelingen obstetrie en erfelijkheidsvoorlichting - op een vrijdagmiddag. Aanmelding dient zo vroeg mogelijk in de zwangerschap, in elk geval voor de 9e week, plaats te vinden. De vlokkentest vindt dan op een dinsdagochtend plaats, transcervicaal, onder echoscopische kontrole. De procedure is pijnloos en neemt 15 à 30 minuten in beslag.

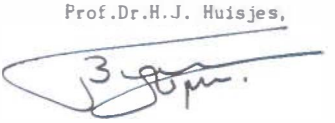
Mocht u meer informatie wensen dan kunt u met de onderstaande, bij de uitvoering van de chorionvillibiopsie test betrokken, personen kontakt opnemen:

Afdeling obstetrie:	drs. A. Mantingh, gynaecoloog
(050-613080)	dr. G.H.A. Visser, gynaecoloog

Afdeling Erfelijkheidsvoorlichting: drs. A.S.P.M. Breed, arts, klinisch
(050-118376) geneticus i.o.
mw.dr. L.C.P. Govaerts, kinderarts,
klinisch geneticus i.o.

Met collegiale hoogachting,
namens Prof.Dr.G.J.P.A. Anders en
Prof.Dr.H.J. Huisjes,





A. Mantingh A.S.P.M. Breed

Lit.: Nederlands Tijdschrift voor de Geneeskunde 1984; 128: nr. 10, 436-441.
Human Genetics 1984; 66: 252-259.
Prenatal Diagnosis 1984; 4: 279-287.

11.6 Key-correspondence concerning rejected trial chorionic villus sampling versus amniocentesis in English and Dutch)

DEPARTMENT OF
OBSTETRICS AND GYNECOLOGY

STATE UNIVERSITY
59 OOSTERSINGEL
GRONINGEN
THE NETHERLANDS

GRONINGEN, May 1, 1984

Dr. Bernadette Modell
University College Hospital
Obstetric Hospital
Huntley Street
LONDON WC1E 6AU

Dear Mrs. Modell,

This is to confirm my willingness to take part in a prospective trial concerning chorion villus sampling, as mentioned in Iain Chalmers' letter to you of 10th April. I am not sure how many cases we would be able to contribute. At present we perform about 300 amniocenteses a year, but the crucial point is, of course, how many of them will be referred before the 11th week.

In a few weeks there will be an informal meeting between the chiefs of three major centers for antenatal diagnosis in the Netherlands. If all of them would be willing to contribute, this would increase the number of cases considerably. Please let me know whether it would be worth while to ask them to do so. In that case I would have to have your permission to let them study the protocol.

Yours sincerely,

prof.dr. H.J. Huisjes

DEPARTMENT OF
OBSTETRICS AND GYNECOLOGY

STATE UNIVERSITY
59 OOSTERSINGEL
GRONINGEN
THE NETHERLANDS

GRONINGEN. February 1, 1985

Iain Chalmers, M.D.
RADCLIFFE INFIRMARY
Oxford OX2 6HE
Great Britain

Dear Iain,

Thank you for your letter of 7th January about the CVS trial. I wonder how many we will be able to get into the trial. Here up to now, all those who would have been eligible had made up their mind already for either CVS or amniocentesis. Hopefully it does not turn out not being worth the extra effort for those few we might get in future. In this regard it is a pity Rotterdam is out (why?). Whatever, we are willing to participate, as Henk said before. Our Ethics Committee got their part of the papers, no idea how speedy they are.

I'll let you know, in the meantime I'll keep spirits high by listening to the DSC Jubilee tape I managed to get for you (new!).

Bye,

Albert.

Albert Mantingh,

for Henk Huisjes,

RIJKSUNIVERSITEIT

FAKULTEIT DER GENEESKUNDE
ANTHROPOGENETISCH INSTITUUT

9713 AW GRONINGEN, Antonius Deusinglaan 4
Telefoon 050-116120

21 augustus 1985

Aan de hooggeleerde heer
Prof.dr.H.J. Huisjes,
Vrouwenkliniek
Academisch Ziekenhuis
Oostersingel 59
GRONINGEN

Beste Huisjes,

Ik heb de laatste stukken die je nog over het gerandomiseerde onderzoek m.b.t. chorionbiopsie hebt gestuurd grondig bekeken.
Mijn conclusie is de volgende:

1. van de vier doelen op p.1 /i,ii..... zijn de drie eersten praktisch even goed te bereiken met het WHO Registry van Jackson in Philadelphia.
2. Het vierde doel is bijzonder boeiend en is waarschijnlijk in principe beter te bereiken via de Oxford methode, maar in het Oxford stuk bijzonder zwak onderbouwd.
3. wat het eerste doel betreft, het onderzoek naar de short term effects, is ondanks de onberispelijke statistische aanpak de complexiteit van de situaties die moeten worden vergeleken zo groot, dat de uitkomst niet veel meer informatie zal geven dan van de gewone paralelle registratie kan worden verwacht. Met name de vooronderstellingen betreffende de betekenis van chromosomale afwijkingen bij spontane abortus tussen de 8ste en 16de week zijn bijzonder zwak.
4. De vragen die met de short term results zouden kunnen worden beantwoord p.3 sub 2, i, ii, iii zijn even goed en vlugger met de registry te beantwoorden.

Tegen deze achtergrond vind ik dat de eerste zin bij Ethical considerations: Current ignorance etc erg slecht past. Er is geen sprake van poorly controlled manner bij de gewone registratie, als men ze vergelijkt met de praktische uitkomsten van de randomized trial.

De daarop volgende conclusie betreffende random allocation klinkt dan weinig overtuigend.
Overigens zijn volgens mijn ervaring de meeste zwangeren die voor prenatale diagnostiek bij ons komen sterk gemotiveerd voor een zo vroeg mogelijke ingreep.
Wij hebben om aan deze behoefte te voldoen met grote haast aan het begin van het jaar dit onderzoek in de gehele regio aangeboden o.a. om een dienst verlening ter beschikking te stellen die al in Rotterdam aanwezig was. Wij hebben dat gedaan zonder keuze en bijkomstigheden. Een verandering in deze benadering zal gauw tot misverstanden bij perifere artsen en patienten aanleiding geven. Precies zoals het feit dat ooit jaren geleden een enkel centrum prenatale diagnostiek deed onder de voorwaarde dat de patienten zich verplichtten om abortus consekwenties te aanvaarden. Deze ellende spookt nog altijd door heel Nederland.
Ik geloof dat, gezien de weinig overtuigende achtergrond van de randomisation, het niet verantwoord is in een grotere groep van patienten onrust te brengen om enkelen over te houden die bereid zouden zijn mee te doen.
Het zou ook weinig rendabel zijn het hele voor deze aanpak noodzakelijke informatie apparaat op te zetten.
Maar ik til vooral heel zwaar aan de onrust die deze benadering, die bovendien niet landelijk zal zijn, in patientenkringen zal veroorzaken. Ik ben er zeer voor geporteerd dat we ons bij het WHO Registry van Jackson aansluiten. Ik sluit een fotocopie van een recent gegeven uit dat register bij. Nu, een jaar later, zullen zeker een paar duizend andere gevallen met hun uitkomsten daar bekend zijn.
Ik denk dat deze aanpak voor ons de meest realistische is.

Met vriendelijke groeten,

Prof.Dr.G.J.P.A. Anders

CURRICULUM VITAE

The author was born in Stadskanaal on 7 Januari 1943; attended primary school there and Gymnasium ß in Groningen; completed his medical studies in 1969 at the University of Groningen after a one-year-intership in Curaçao; returned to Curaçao as resident in tropical surgery and obstetrics and left in 1971 for Kenya to work as a medical officer at the Homa Bay District Hospital, South Nyanza; was short-term Ford Foundation consultant in Hong Kong (re. funding and policy-making of reproductive sciences and contraceptive development); started specializing in obstetrics and gynaecology in 1974 in Groningen and left again for Kenya in 1980 to work as a provincial gynaecologist at the Machakos Hospital, Eastern Province and as lecturer/external examiner at the University of Nairobi; returned in 1983 to the department of Obstetrics and Gynaecology, University Hospital, Groningen and is presently in charge of the Antenatal Diagnosis Unit; is married to a lovely wife (Irene), has two naughty boys (Martijn and Rogier) and can type amazingly fast with two fingers.