



WHO

REGIONAL OFFICE FOR EUROPE

EUR/ICP/MCH 123 (A)

00265

ENGLISH ONLY

UNEDITED

J3705

CHORIONIC VILLUS SAMPLING (CVS) SAFETY

Report of WHO/EURO Meeting
In association with the 7th International
Conference on Early Prenatal Diagnosis
of Genetic Diseases

Tel-Aviv
21 May 1994

SCHERFIGSVEJ 8
DK-2100 COPENHAGEN Ø
DENMARK

TEL.: (45) 39 17 17 17
TELEFAX: (45) 39 17 18 18
TELEX: 12000

1995

EUR/HFA target 4

TARGET 4

REDUCING CHRONIC DISEASE

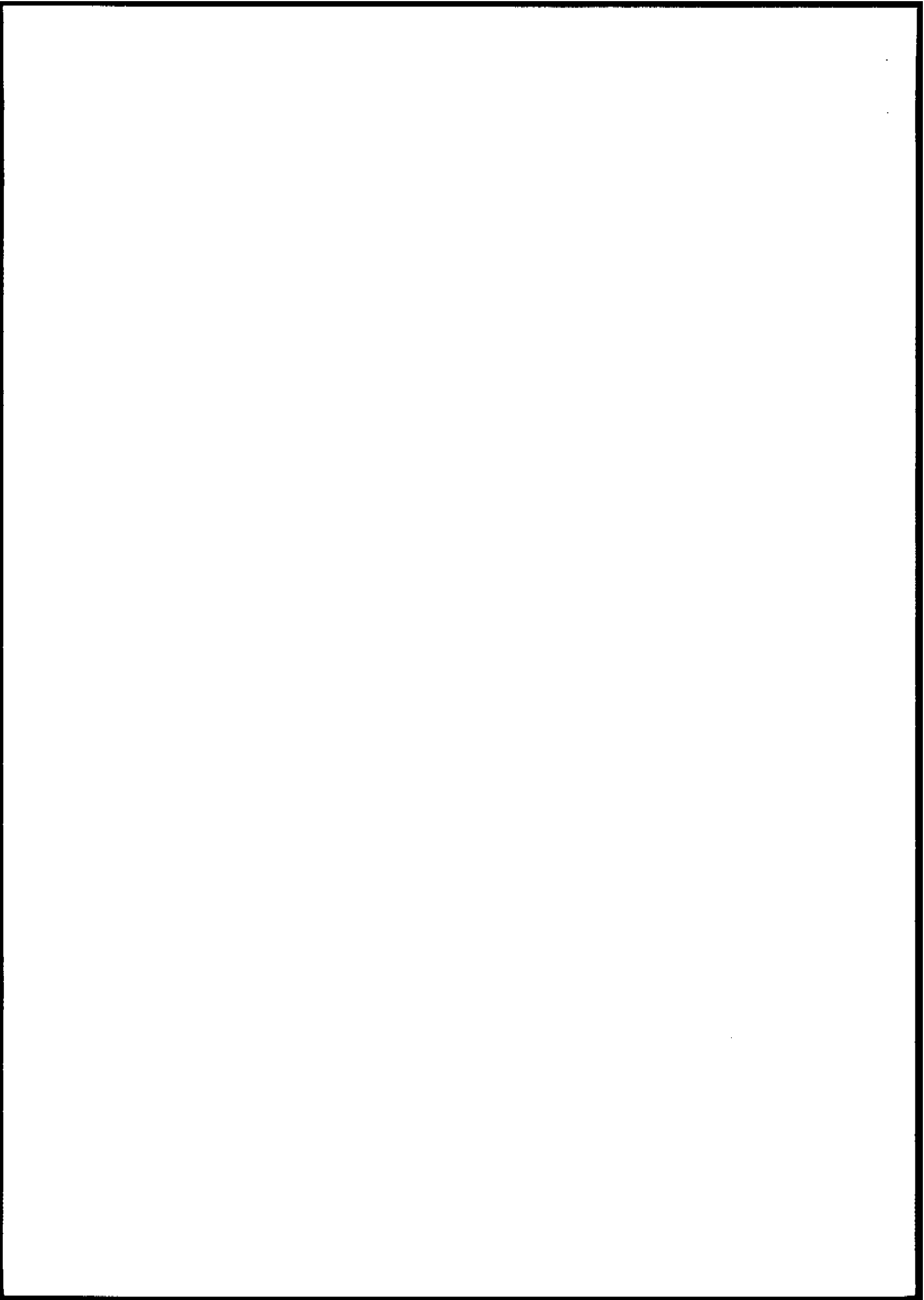
By the year 2000 there should be a sustained and continuing reduction in morbidity and disability due to chronic disease in the Region.

Keywords

CHORIONIC VILLUS SAMPLING
PRENATAL DIAGNOSIS
GENETIC SCREENING
RISK

Table of Contents

1.	Introduction.....	2
2.	Fetal loss rates and prevalence of LRD.....	2
2.1	Fetal loss rate as an indicator of CVS performance.....	3
2.2	Limb reduction defects.....	3
3.	Comparative Pattern Analysis of LRD in the General Population and after CVS.....	4
4.	Conclusions and Recommendations	6
5.	Participants.....	7
6.	References.....	7



Chorionic Villus Sampling Safety

Report of a WHO/EURO Meeting in Association
with the 7th International Conference on Early Prenatal Diagnosis
of Genetic Diseases
Tel-Aviv, 21 May 1994

1. Introduction

Chorionic villus sampling (CVS), introduced in the early eighties, is now an established medical procedure in the first trimester of pregnancy. The global experience would be difficult to evaluate, as hundreds of centers around the world perform CVS, but only 146 have consistently reported their cases to the WHO sponsored CVS registry in Philadelphia. A total of 158,774 CVS have been registered to date, providing a valuable source of data for analysis of pregnancy outcomes following CVS. Because of a few reported clusters of children born with limb anomalies after CVS, a previous WHO/EURO 1992 meeting (1) examined collected data on 80 000 cases available at that time and made a statement on the safety of CVS and its use in prenatal diagnosis. Although there was no strong evidence for an increased risk of fetal loss and congenital malformations associated with CVS, it was recommended that follow-up data be collected on the short- and long-term complications of CVS, including limb reduction defects (LRD). A special format for reporting such cases was published to facilitate standardizing data collection and also to re-evaluate previously reported material. Because there were not enough data for accurate evaluation of the effect of early CVS, it was recommended that CVS should be performed only after 8 weeks' gestation.

The present WHO/EURO follow-up meeting was organized in association with the 7th International Conference on Early Prenatal Diagnosis of Genetic Diseases. The major objective was to analyze the prevalence and the pattern of LRD in the registry material and to make recommendations regarding the future use of CVS in clinical practice.

2. Fetal loss rates and prevalence of LRD

The CVS Registry has been maintained since 1983, and as noted now includes data from 146 centers, each of which has registered from as few as 100 to as many as 13,500 CVS. A total of 158,774 cases from all centers were registered up to May 1994, with data available on pregnancy loss and infant outcome. Some smaller volume centers who have not revised their figures in some time and did not respond to recent inquiries for updates and special details of congenital malformations (including LRD) have not been retained in the Register, unless they registered at least one case of LRD. Thus no case of LRD has been discarded, and the registry figures may actually overestimate the rate of LRD.

2.1 Fetal loss rate as an indicator of CVS performance

Surveillance of fetal loss rates was one of the major objectives of the CVS registry from the very beginning. Fetal loss rate was among the criteria recommended by the WHO/EURO 1992 meeting (1) for self-evaluation of the expertise of centers (expertise is estimated to be usually achieved with experience of about 250 procedures and maintained by systematic sampling). Among the currently active registry participants the rate of spontaneous pregnancy loss following CVS averages 2.5-3.0%, with several large volume operators having the loss figures at or below 2%. These figures can be considered illustrative of the expected performance from well-established CVS programs having volumes of several hundred cases per year, and will produce procedure associated loss rates comparable to amniocentesis loss rates. Randomized comparisons of CVS and amniocentesis, including those programs which analyzed CVS and amniocentesis at comparable gestational age, have confirmed this observation (2-4). A recent comparison of fetal loss after CVS and amniocentesis performed at similar gestational age (10-13 weeks) showed a significantly higher loss rate of early amniocentesis (5). Newer programs with smaller volumes and less experience would be expected to have slightly higher aggregate fetal loss rates, as seen from MRC international study, that included a large number of centers that contributed only a few patients (6).

Since the appearance of clusters of LRD in some centers has coincided with fetal loss rates considerably higher than the figure given above, the question of the possible association of the two occurrences has been raised (81). Fetal loss rates outside the expected range should prompt a search for factors which might contribute to safe performance. Because fetal loss rate appeared to be a useful criterion for evaluating the expertise of a center, the WHO registry continues surveying its contributing centers for current updates of these figures. The baseline overall fetal loss rates in 158,774 procedures reported, are not different from those documented above.

2.2 Limb reduction defects

Incidence figures for LRD in various population-based studies range from 4.8/10,000 to 5.97/10,000 (7-11). Of 158,774 cases with completed follow-up reported to the CVS registry, there were 77 LRD among completed pregnancies (including spontaneous abortions and stillbirths). The 4.9/10,000 incidence among the registered cases after CVS is well within the usual population limits. It is also well established that the incidence of LRD among stillbirths and pre-viable fetuses is far higher up to 39.52/10,000 among stillbirths (12). If no distinction is made between liveborn and stillborn cases, as well as terminated fetuses and spontaneous abortions, the mixed population being evaluated would be expected to show a much higher overall incidence of LRD (13). Although the registry data included 4 terminated cases with LRD, the incidence following CVS is still not different from the baseline data.

Although reports to the CVS registry are mainly voluntary, a special effort has been made to seek out and include known "clusters" of LRD despite the lack of

direct reporting through the normal mechanism. For example, 84,600 cases have been listed from US centers, including 33 LRD; this rate of 3.9 per 10,000 is well within the expected limits. These cases include 2 centers, both of which are listed only because they have reported LRD (14,15). This again would bias the data toward an excess of observed LRD. In the US group there are 2 centers with less than 500 cases, 9 centers with 1-2000 cases, 5 with 2-3000, 4 with 3-5000, 2 with 5-6000 and 2 large programs with more than 13,000 CVS. With the exception of the two cluster centers, both fetal loss rates and the frequency of LRD in these centers are uniformly low.

3. Comparative Pattern Analysis of LRD in the General Population and after CVS

A meaningful comparison of the results obtained in various cohorts of patients with LRD is only possible if a well-defined and reproducible approach and classification is used. Therefore, the pattern distribution of LRD following CVS was compared to the data obtained in the population based British Columbia study, which covered a 30-year observational period, using the same definition of LRD, the same guidelines for excluding the cases, and the same classification and pattern analysis (7,16).

The following LRD cases listed in the CVS registry were excluded from the pattern analysis. Ten were actually not LRD, 6 represented syndromes or genetic conditions, 1 had amniotic band sequence with limb involvement, 7 presented with hypoplastic nails only, and 4 were therapeutic termination of pregnancy because of severe malformations found on ultrasound examination. The case with amniotic band sequence was excluded to be consistent with the pattern analysis in British Columbia study (16-17). The etiology of amniotic band sequence is not clear and it has been suggested that it might represent a very early defect of the organization of the embryo, prior to 26 days after conception (40 days after last menstrual period) (18). The 4 cases in which termination of pregnancy had been performed were also excluded because it cannot be predicted that these cases with severe malformations would have survived to term; thus, these cases cannot be compared to data from a liveborn population. Cases with nail hypoplasia only were excluded because minor anomalies can be expected to be underestimated in population based studies (19). Thus, ascertainment had been different with respect to these cases between the British Columbia and the CVS data, where special interest was paid to terminal defects of the digits and toes.

To analyze the patterns of LRD, cases were classified in a hierarchic system, related to the development of the limb, as described in the British Columbia study (16). This gives 5 subgroups for upper limb defects and 5 major subgroups for defects affecting only the lower limb. As there were no CVS cases with defects in some of the categories, it was sufficient to use a total of the following seven different subgroups:

1. Amelia (total absence of one or more limbs from the shoulder or pelvicgirdle).

2. Defects of the ulna.
3. Defects of radius/ulna (i.e. transverse defects from the elbow to the wrist).
4. Defects of the hand.
5. Defects of the digits.
6. Defects of the femur.
7. Defects of the toes.

Each case was counted only once, according to the defect which was superior in the hierarchy. Thus, for example, a case with defects of one hand and also defects of one foot would only appear in the category 5 for hand defects and would not be recounted for foot defects. This approach was taken to be consistent with the analyzing system used in the British Columbia Study (16). All cases with LRD in the CVS cohort were compared to the corresponding subgroups of defects from the population-based data.

The pattern analysis of limb defects occurring in the CVS cohort showed no statistically significant differences compared to the background data. Upper limbs were involved in 33 cases (67.3%), the lower limbs in 6 cases (12.2%) and both upper and lower limbs in 10 cases (20.4%). Four cases involved on the right side (8.16%), 23 only the left side (46.9%), in 5 cases (10.2%) the defect was unilateral, but no further specified, and in 17 cases defects were bilateral (34.7%). There were 20 cases with transverse defects (excluding the case of amelia) (42.2%) and 29 cases with longitudinal defects (59.2%), compared well to the background population data (Fig.1). Furthermore, the pattern of LRD in the all other subgroups showed no statistically significant differences, if compared to the corresponding subgroups from the background population.

Only one of the liveborn cases was in the subgroup with total absence of limb (amelia), i.e. 2.04% of the total of 49 analyzed LRD, compared to 3.19% in the general population. The rates for hand and finger defects were higher than in the general population. However, these are the most frequent forms of nonspecific limb anomalies and the frequency of these two groups was not statistically significant from their frequency in the background data (16). The subgroup of toe defects, by contrast, was smaller than in the comparable group in the general population.

One caveat is that the total number in the CVS group is small: the total number in the CVS cohort with LRD constitutes only 10% of the total number of limb deficiencies in the general population study. This applies especially for those subgroups with only single cases (amelia, femur, ulna). It is also noticeable that there were no cases in the groups of longitudinal radius defects, preaxial ray defects of the upper limb or humerus defects. All cases with longitudinal radius defects or preaxial defects of the upper limbs (thumb defects) in the present cohort had these limb anomalies as part of a syndrome or general condition (Fanconi anaemia, VACTERL-hydrocephalus syndrome, Holt-Oram syndrome). The only case with a humerus defect was the one with amniotic band sequence. It is further of interest that the majority of case with amelia were among the terminated pregnancies, with one case representing a limb-body-wall complex, one case of lower limb amelia and trisomy 3 mosaicism, and a further case of bilateral symmetric amelia of the upper limbs with twisted short tibia, micrognathia and a short neck. The last two cases

were included in the series published by Mastroiacovo and coworkers (19), who considered the second one to be an example of Hanhart syndrome. This is disputable because the twisted tibia and further dysmorphic features are not commonly seen in this syndrome. Finally, two cases with involvement of radius and ulna were carriers of a balanced Robertsonian translocation (t (13;14)).

These results show neither an increase in the overall incidence of LRD, nor any difference in the pattern of deficiencies compared to pattern subgroups of limb defects in the general population. This was particularly relevant to transverse limb defects, which show no increased risk after CVS. The data provide no evidence for any specific risk of limb defects caused by CVS.

Early CVS. Too few data exist to analyze a potential causal relationship between LRD and early CVS, as only a small proportion of the registered CVS have been performed earlier than 9 weeks of gestation.

Firth et al. (20), in a literature review collected 75 LRD in babies exposed to CVS, with the purpose of analyzing the temporal relation between CVS and LRD. The authors claimed a possible inverse correlation between severity of defect and gestational age. In 4 of the included cases, the defects listed were not LRD according to the definition used in the population-based studies. Further, in 18 cases, the gestational age was estimated as the mean of a 14 day range, making these cases essentially useless for the intended purpose of the study. Finally, it has been established that by ascertaining these cases from literature reports there has been unknowing duplication of several cases further undermining the conclusion. Similar difficulties of ascertainment as well as methodological errors have raised criticism of the CDC study reported by Olney and others (21). Again, these misclassifications move findings of borderline significance into the non-significant range.

Although most centers have stopped offering CVS earlier than 9 weeks, efforts should be encouraged to register all cases from those communities in which CVS is not acceptable beyond 8 completed weeks, so that a representative CVS sample could be obtained for prevalence and pattern analysis of LRD following early CVS. As mentioned, such studies should use the generally accepted classification, and standardized data collection, to be able to compare with population based studies and to identify possible genetic and environmental causes of LRD. For example, an important causal relationship has been recently demonstrated between isolated terminal transverse limb defects and maternal smoking (22). This and other known environmental exposures may lead to some of the reported clusters of LRD (23).

4. Conclusions and Recommendations

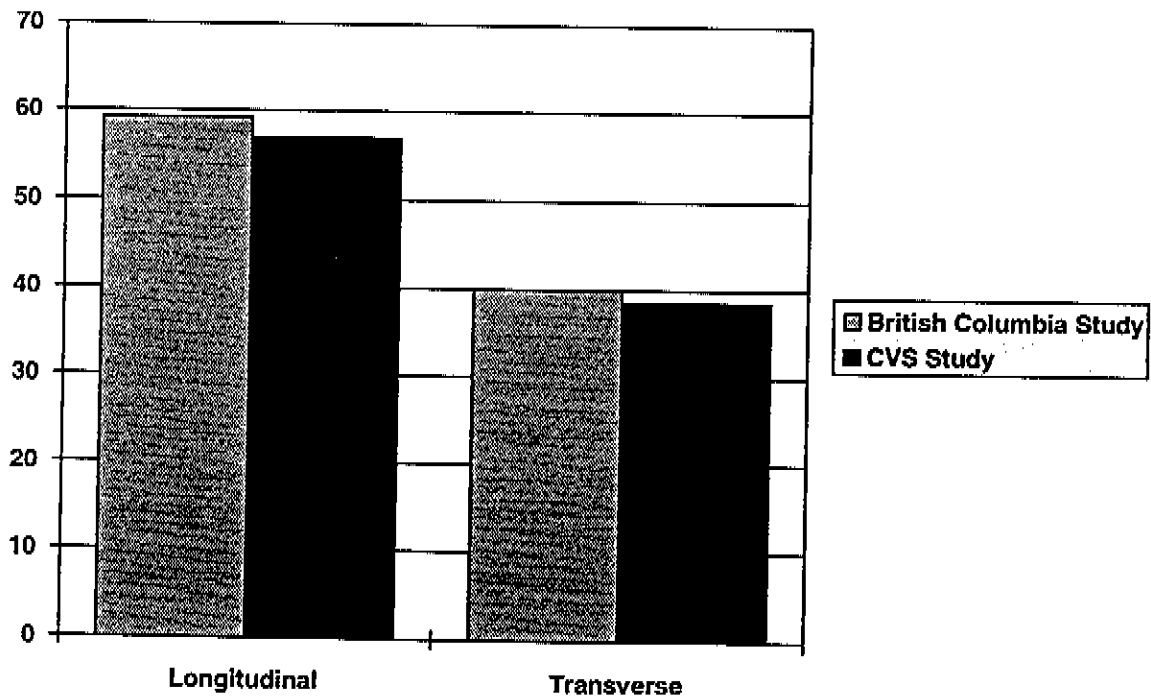
Accumulated experience of 158,774 CVS cases shows that CVS is a safe procedure with an associated fetal loss rate comparable to amniocentesis. The CVS registry shows that CVS is currently performed mainly between 9 and 12 weeks' gestation and carries no increased risk of LRD: the overall incidence of LRD after CVS is 4.9 per 10,000 CVS, compared to the range from 4.8/10,000 to 5.97/10,000 in the general population.

Analysis of the pattern distribution of limb defects after CVS revealed no difference from the pattern of limb defects in the general population. This applies specifically to transverse limb defects. Together with the overall incidence of LRD, these data provide no evidence of any risk for congenital malformation determined by CVS.

As CVS is currently performed generally after 8 completed weeks of pregnancy, few data are available for analysis of complications related to earlier procedures. Avoiding early CVS also excludes sampling in cases of early fetal death, which can be diagnosed reliably by ultrasound at 9 weeks of pregnancy.

The data collected in the CVS registry shows the value of continuing large scale prospective surveys of possible maternal and fetal risks of invasive procedures in pregnancy, as no centers' individual experience can provide enough data to establish or exclude causal relationships.

Fig. 1 Comparison of limb defects - Longitudinal and transverse



5. Participants

Bruno BRAMBATI - Italy
Ursula FROSTER - Germany, Rapporteur
Norman GINSBERG - USA
Laird JACKSON - USA, Rapporteur
Anver Kuliev - WHO representative, Chairman
Joe Leigh SIMPSON - USA
Steen SMIDT-JENSEN - Denmark
Yury VERLINSKY - USA
Haim ZAKUT - Israel

6. References

1. Risk evaluation of CVS. WHO/EURO Document EUR/ICP/MCH 123 (1992) Copenhagen, WHO Regional Office for Europe.
2. Smidt-Jensen, S., Permin M., Philip, J. (1991). Sampling success and risk by transabdominal chorionic villus sampling, transcervical chorionic villus sampling and amniocentesis. *Ultras Obstet Gynecol* 1:86-90.
3. Canadian Collaborative CVS-Amniocentesis Trial Group (1992). Multicentre randomised clinical trial of chorionic villus sampling and amniocentesis: final report. *Pren Diagn* 12: 385-476.
4. Palo, P., Piironen, O., Honkonen, E., Lakkala, T., Aula, P. (1994) Transabdominal chorionic villus sampling and amniocentesis for prenatal diagnosis: 5 years' experience at a University centre. *Pren Diagn* 14: 157-162
5. Nicolaides, K., Brizot, ML., Patel, F., Snijders, R. (1994) Comparison of chorionic villus sampling and amniocentesis for fetal karyotyping at 10-13 weeks' gestation. *Lancet*, 344: 435-439.
6. MRC Working Party on the Evaluation of Chorionic Villus Sampling. Medical Research Council European Trial of chorionic villus sampling. 1991. *The Lancet* 337: 1491-9.
7. Froster, U.G., Baird, P.A. 1989. Limb reduction defects in over one million consecutive live births. *Teratology* 39: 127-35.
8. Aro, T., Heinonen, O.P., Saxen, L. (1982). Incidence and secular trends of congenital limb defects in Finland. *J. Epidemiol* 11: 239-244.
9. Kallen, B., Rahmani, T.M., Winberg, J. (1984) Infants with congenital limb reduction registered in the Swedish Register of congenital malformations. *Teratology* 29: 73-85.

10. Carsolary E., Manservigi, D., Carani, G.P., Magnani, C., Milan, M. (1990). Limb reduction defects in Emilia Romagna, Italy: epidemiological and genetic study in 173,109 consecutive births. *J. Med Genet* 27: 353-357.
11. Evans, J.A., Vitez, M., Czeizel, A. (1994). Congenital abnormalities associated with limb deficiency defects: A population study based on cases from Hungarian Congenital Malformation Registry. *Am J Med Genet* 49: 52-66.
12. Froster, U.G., Baird, P.A. (1993). Congenital defects of the limbs in stillbirths: Data from a population-based study. *Am J. Med Genet*: 46: 479-82.
13. Stoll, C., Alembik, Y., Roth, M.P. (1992). Risk factors in limb reduction defects. *Paediatr Perinat Epidemiol* 6: 323-338.
14. Burton, B.K., Schulz, C.J., Burd, L.I. 1992. Limb anomalies associated with chorionic villus sampling. *Obstetrics and Gynaecology* 79: 726-30.
15. Bissonette, J.M., Busch, W.L., Buckmaster, J.G., Nesslet, C.J. (1993). Factors associated with limb anomalies after chorionic villus sampling. *Prenat Diag* 13: 1164-1165.
16. Froster, U.G., Baird, P.A. (1992) Upper limb deficiencies and associated malformations: A population-based study. *Am J Med Genet* 44: 767-781.
17. Froster, U.G., Baird, P.A. (1993) Amniotic band sequence and limb defects. *Am J Med Genet* 46: 497-500.
18. Bamforth, J.S. (1992) Amniotic band sequence: Streeter's hypothesis reexamined. *Am J Med Genet* 44: 280-287.
19. Mastroiacovo, P., Botto, L.D., Cavalcanti, D.P., Lalatta, F., Selicorni, A., Tozzi, A.E. (1992). Birth defects following chorionic villus sampling. *Am J Med Genet* 44: 856-864.
20. Firth, H.V., Boyd, P.A., Chamberlain, P., MacKenzie, I.Z., Lindenbaum, R.H. and Huson, S.M. (1994). Analysis of limb reduction defects in babies exposed to chorionic villus sampling. *Lancet* 343: 1069-1071.
21. Olney, R., Khouri, M.J., Botto, L.D., Mastroiacovo, P. (1994). Limb defects and gestational age at chorionic villus sampling. *Lancet* 344: 476.
22. Czeizel, A., Koda, I., Lenz, W (1994). Smoking during pregnancy and congenital limb deficiency. *BMJ* 308: 1473-1476.
23. Van Den Anker, J.N., Van Vught, E.E., Zandwijken, G.R.G., Cohen-Overbeek, T.E., Lindhout, D. (1993). Severe limb abnormalities: Analysis of a cluster of five cases born during a period of 45 days. *Am J Med Genet* 45: 659-667.