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# Chorionic villus sampling

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Chorionic villus sampling has gained importance as a tool for early cytogenetic diagnosis with a shift toward first trimester screening. First trimester screening using nuchal translucency and biomarkers is effective for screening. Chorionic villus sampling generally is performed at 10-12 weeks by either the transcervical or transabdominal approach. There are two methods of analysis; the direct method and the culture method. While the direct method may prevent maternal cell contamination, the culture method may be more representative of the true fetal karyotype. There is a concern for mosaicism which occurs in approximately 1% of cases, and mosaic results require genetic counseling and follow-up amniocentesis or fetal blood sampling. In terms of complications, procedure-related pregnancy loss rates may be the same as those for amniocentesis when undertaken in experienced centers. When the procedure is performed after 9 weeks gestation, the risk of limb reduction is not greater than the risk in the general population. At present, chorionic villus sampling is the gold standard method for early fetal karyotyping; however, we anticipate that improvements in noninvasive prenatal testing methods, such as cell free fetal DNA testing, will reduce the need for invasive procedures in the near future.

**Key words:** Chorionic villus sampling (CVS).

## Introduction

Two major diagnostic tools for fetal karyotyping are first trimester chorionic villus sampling (CVS) and second trimester amniocentesis. The advantages of earlier diagnosis include reduced maternal risk associated with termination of pregnancy, and a lessened emotional burden on the woman. The shift toward first trimester screening for Down syndrome has increased the importance of CVS in contemporary practice. CVS came into general use in the 1980s with the use of ultrasonography-guided techniques, and the refinement of the sampling catheter. There have been issues related to the safety of CVS, compared with mid-trimester amniocentesis, and concerns regarding the interpretation of results, primarily in cases with mosaicism. Currently, noninvasive prenatal testing (NIPT) is emerging as an effective method of screening for aneuploidy. This review briefly summarizes contemporary issues relating to the use of CVS.

## Counseling for Aneuploidy Screening or Invasive Diagnostic Testing

The American College of Obstetricians and Gynecologists (ACOG) has recommended that screening and invasive diagnostic testing for aneuploidy should be available to all women, regardless of maternal age [1,2]. Every women should be counseled nondirectively on each screening and diagnostic

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option, in order to enable them to reach their own decisions.

Table 1 lists patient characteristics associated with an increased risk of fetal aneuploidy [1]; their risk factors constitute the indications for CVS. Relative contraindications to CVS are listed in Table 2 [3].

Assessment of nuchal translucency and biochemical markers in the first trimester provides an effective method of screening for Down syndrome in the general population; its screening efficacy is comparable to that of the quadruple test [4]. Integrated first and second trimester screening shows more sensitive results, with a lower false positive rate. Major fetal anomalies detected during the second trimester ultrasonography warrants counseling, and the offer of a diagnostic procedure. Down syndrome screening in multiple gestation is less accurate, and first trimester nuchal translucency screening in twins or triplets is feasible, but has a lower sensitivity [2].

Cell free fetal DNA testing has emerged as a promising screening tool for Down syndrome, with an expected sensitivity of >98%, and a false positive rate of <0.5%; the method is, however, associated with higher costs [5,6]. Although cell free fetal DNA testing is now available to high-risk women as a screening test, it does not replace the diagnostic accuracy of CVS or amniocentesis, as false positive and false negative test results can occur. Invasive procedures still remain the gold standard tests for fetal karyotyping, but with an accompanying small risk of pregnancy loss.

**Table 1.** Patient characteristics associated with an increased risk of fetal aneuploidy

| |
|---|
| Previous fetus or child with an autosomal trisomy or sex chromosome abnormality |
| One major or at least 2 minor fetal structural defects identified by ultrasonography |
| Parental carrier of chromosomal translocation or chromosomal inversion[a] |
| Parental aneuploidy or mosaicism for aneuploidy |

[a]Inversion 9, which is a common variant in the general population, is an exception.

**Table 2.** Relative contraindications to chorionic villus sampling

| |
|---|
| Some active cervical infections, e.g., chlamydia or herpes[a] |
| Vaginal infection[a] |
| Vaginal bleeding or spotting |
| Extreme uterine ante- or retro-version |
| Maternal body habitus precluding easy uterine access or clear ultrasonographic visualization |

[a]Contraindication to transcervical chorionic villus sampling.

## Procedures

CVS generally is performed at 10-12 weeks gestation, when the gestational sac does not yet fill the uterine cavity, and is surrounded by the thick chorionic membrane. The chorionic villi at the implantation site proliferate to form the chorion frondosum, which is in contact with the decidua basalis. During these weeks, the chorionic villi are loosely anchored to the decidua basalis, and float freely in the intervillous space.

### 1. Transcervical CVS technique (Fig. 1)

The positions of the uterus and placenta are confirmed by ultrasound examination; adequate bladder filling may enhance ultrasound visualization, while overfilling may push the uterus upwards. The patient is placed in the lithotomy position and the vulva and vagina are prepared aseptically. A vaginal speculum is inserted and the cervix is prepared with povidone iodine solution. A thin polyethylene catheter with a round-tipped malleable stylet is shaped into a slight curve, and gently inserted through the cervical canal under ultrasound guidance. The assistant adjusts the position of the ultrasound probe to visualize the catheter tip. The catheter is advanced along the placenta. The stylet is removed, and a 20 mL syringe containing tissue culture medium is connected; the catheter is then removed slowly, with negative pressure. The syringe is visually inspected for the presence of branching tissues floating in the media, sometimes with the aid of a low-power dissecting microscope. If the retrieved villi are insufficient, a second insertion is carried out with a new catheter.

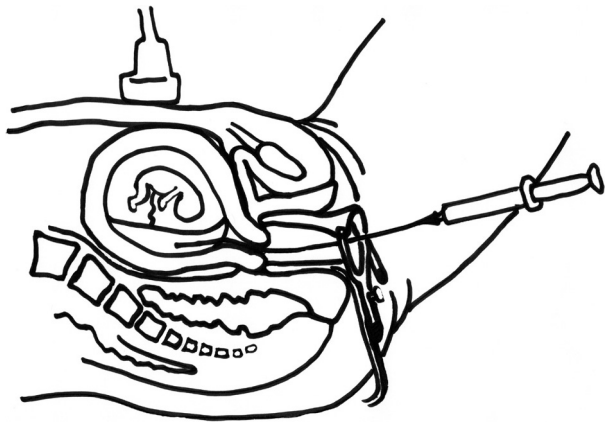

**Fig. 1.** Transcervical chorionic villus sampling technique.

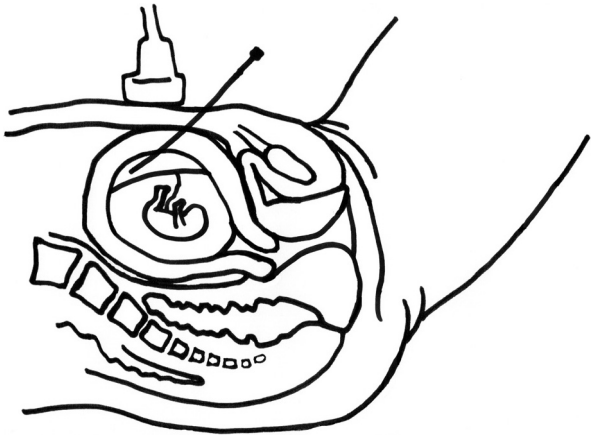

**Fig. 2.** Transabdominal chorionic villus sampling technique.

**2. Transabdominal CVS technique (Fig. 2)**

The positions of the uterus, placenta, and bowel are confirmed by ultrasound examination. The abdomen of the patient is aseptically prepared with povidone iodine solution. Under continuous ultrasound guidance an 18 or 20 gauge spinal needle is inserted into the placenta. Care must be taken to avoid puncturing the bowel. Tissue is retrieved using negative pressure and 3-4 back and forth movements.

When performing CVS in a twin pregnancy, each placental site must be distinguished and sampled separately. This requires a meticulous ultrasound examination and a detailed topographic description with drawings. Separate tissue retrieval, using a transabdominal approach for one sample and a transcervical approach for the other, may minimize the risk of cross-contamination. A backup amniocentesis may be considered if there is a suspicion of inadequate sampling with concordant fetal sex.

Anti-D immune globulin is administered to Rh-D negative unsensitized patients [7]. Neural tube defect screening is carried out during the second trimester, using either ultrasound or alpha-fetoprotein measurement. Although CVS may result in a transient elevation of maternal serum alpha-fetoprotein level, it returns to the normal level by the time of second trimester maternal serum screening [8].

## Laboratory Aspects

The obtained sample contains 5-30 mg of villous material. Villous tissue is carefully separated from any adherent decidua under a dissecting microscope, and it is treated with trypsin to separate the cytotrophoblasts from the mesenchymal core of the villi. Cytogenetic analysis can be performed using either the direct method or the culture method. The direct method analyzes actively dividing cytotrophoblasts, and provides rapid results (within 2 days) while minimizing the risk of maternal cell contamination. In some classification systems, it may be subdivided into the direct method, in which same-day analysis is performed, and the short-term culture method, in which analysis is performed within a next day or 2. The culture method, also called the indirect or long-term culture method, analyzes the mesenchymal core of the villi, and provides results within 6-8 days. The culture method, which analyzes the mesenchymal core, may represent the fetal karyotype more correctly than analysis of the cytotrophoblast, because the mesenchymal core of the villi is genealogically closer to the embryo, and any trace trophoblast cells disappear in a few days during culture. However, maternal decidual cells may grow in the culture, resulting in the potential for diagnostic errors. The direct method of trophoblast analysis may prevent maternal cell contamination, because the decidua has a low mitotic index. Maternal cell contamination can be minimized by obtaining an adequate amount of villous tissue, and selecting only typical villous material while discarding atypical fragments. Ideally, CVS samples should be analyzed by both the direct and culture method.

In addition to the traditional method of karyotyping, various analytic methods are available, including metabolic or biochemical analysis, and molecular methodology, for example fluorescence *in situ* hybridization (FISH), quantitative fluorescence polymerase chain reaction (QF-PCR), DNA sequencing, and comparative genomic hybridization (CGH) [9, 10].

## Mosaicism

Mosaicism is observed in approximately 1% of CVS samples; however, in the majority of cases the mosaicism in the CVS does not reflect a true mosaicism of the embryo. The presence of aneuploid cells in the placental tissues, with a euploid embryo, is described as confined placental mosaicism. In cases where the abnormality arose during tissue culture, despite the embryo and placenta being euploid, it is called pseudomosaicism. The presence of aneuploid cells in both the embryo and the placenta is called generalized mosaicism.

During early embryogenesis, at the 64-cell blastocyst stage, the majority of cells are trophoblasts; approximately 16 cells constitute the inner cell mass, and within this, about 4 epiblast cells develop to form the embryo. Mitotic errors during cell division may produce mosaic cells, and the extent of aneuploidy is dependent on the timing of these division errors. Involvement of the fetus depends on a chance distribution of aneuploid cells. In some cases of mosaicism, one of the trisomic cells that originated from the initial meiotic error may lose one chromosome during subsequent mitotic divisions to form the euploid disomy cell lines, and the embryo may be rescued. This situation may lead to uniparental disomy when the remaining homologous chromosomes are from the same parent.

In terms of laboratory result, a concept of different levels of *in vitro* mosaicism, originally developed for amniocentesis [11,12], may be applied to CVS. In level I mosaicism, a single abnormal cell is seen. With near certainty this is cultural artifact (pseudomosaicism), and it is not reported. In level II mosaicism, 2 or more cells with the same chromosomal abnormality, in a dispersed culture from a single flask are seen; alternatively, a single abnormal colony is present in an *in situ* culture (i.e., probably a single clone). This is almost always pseudomosaicism, and is not usually reported, except in specific circumstances, such as if additional studies are inadequate, or if fetal anomalies were identified. In level III mosaicism, 2 or more cells with the same chromosomal abnormality are distributed over 2 or more independent cultures. This is likely to reflect a true mosaicism, and is reported to the physicians. On rare occasions, mosaicism may be missed, because realistically, only a limited number of cells can be karyotyped.

Mosaicism identified in the cytotrophoblast but not in the mesenchymal core of the villi is usually confined placental mosaicism. Mosaicism identified in the mesenchymal core of the villi may be confined or generalized. A CVS mosaic result requires follow-up by amniocentesis or fetal blood sampling. Cells obtained from amniocentesis originate from the epiblast of the inner cell mass, and are therefore more closely related to the embryo than cells obtained from CVS. However, false positive and false negative amniocentesis results may occur. Detailed ultrasonography is also necessary. Confined placental mosaicism may be associated with pregnancy complications such as fetal growth restriction and stillbirth; mostly, confined placental mosaicism of meiotic origin, usually involving chromosomes 16 and 22, is associated with pregnancy complications [13].

There are practical difficulties in counseling patients about the implications of the mosaic result. There may be a paucity of relevant published cases, and the available studies often have a limited period of follow-up. Therefore, rather than providing a firm answer, the information may serve as a basis for discussion and counseling. There is a concern that some abnormal cell lines may be in an inaccessible organ, such as brain, which cannot be confirmed by usual samples, such as blood and skin. Every case of mosaicism is unique in terms of the extent, distribution, and nature of the abnormal cell lines.

## Safety

The overall pregnancy loss rate after CVS is higher than the rate of loss following mid-trimester amniocentesis; this is considered to be secondary to the higher background rate of spontaneous pregnancy loss in the first trimester. Procedure-related pregnancy loss rate for CVS appears to approach, or equal, the rate of loss for mid-trimester amniocentesis when undertaken in experienced centers [1,14]. The risk associated with amniocentesis at 15-18 weeks gestation is approximately 0.25-0.50% (1/400-1/200); the miscarriage risk from CVS is approximately 0.5-1.0% (1/200-1/100) [15]. Mujezinovic and Alfirevic [16] reported the loss rate within 14 days of CVS and amniocentesis to be 0.7% and 0.6%, respectively; the corresponding loss rate before 24 weeks was 1.3% and 0.9%. The risk may increase with multiple catheter insertions. The transcervical and transabdominal approaches show no different results, although vaginal bleeding is more common following transcervical CVS. Other complications include amniotic fluid leakage and infection. Because there is a learning curve in the performance of CVS, the centralization of this procedure has merits in terms of safe performance and effective training [17].

Agarwal and Alfirevic [18] reported overall pregnancy loss rates for twins following CVS and amniocentesis to be 3.84% and 3.07%, respectively; the corresponding rates for pregnancy loss before 24 weeks were 2.88% and 2.54%. However, there was no data from randomized studies in their systematic review. They estimated a similar overall pregnancy loss rate for CVS and amniocentesis, with an excess risk of about 1% above the background risk.

There is limited information relating to the risk of vertical transmission of invasive procedure in women chronically infected with hepatitis B or C, or human immunodeficiency virus [19]. It would be prudent to discuss noninvasive screening options. There are also few data on the use of transcervical CVS

in women with active herpes infection, although in practice most clinicians would delay the procedure until the woman is asymptomatic.

Some reports [20,21] have suggested an association between CVS and limb reduction defects or oromandibular-limb hypogenesis; abnormalities were correlated with early CVS, performed around 7 weeks gestation. The overall risk of limb reduction from CVS is 0.03-0.10% (1/3,000-1/1,000), which is not significantly different from that in the general population, and the risk of limb reduction appears to be associated with the timing of CVS (<10 weeks, 0.20%; ≥10 weeks, 0.07%) [22]. When the procedure is performed after 9 weeks gestation, the risk of limb reduction is no greater than the general population risk [1].

## Noninvasive Prenatal Testing and Chorionic Villus Sampling

In recent years, cell free fetal DNA testing has become clinically available as a form of NIPT, with the advances in more efficient technology, such as massively parallel genomic sequencing. This test can be performed as early as 10th week of gestation, and provides information on trisomy 13, 18, and 21 within a week. Analysis on archived samples shows a detection rate >98%, and a very low false-positive rate (<0.5%); as of now, this test seems to be the most effective method of screening for aneuploidy in high-risk women. Results of a joint committee opinion of the ACOG and the Society for Maternal-Fetal Medicine indicated that NIPT can be an option for aneuploidy screening in high-risk women, if it is an active, informed choice, after adequate counseling regarding the limitations [5].

Indications for NIPT include women aged 35 years or older; fetuses with ultrasonographic findings that indicate an increased risk of aneuploidy; women with a history of a child affected with a trisomy; a parent carrying a balanced Robertsonian translocation with increased risk of trisomy 13 or trisomy 21; and women with a positive first or second trimester screening test result [5,6].

There are some limitations to the use of NIPT. First, currently the test only gives information on common trisomies, although some efforts are being made to detect other genetic abnormalities. Second, NIPT is currently a screening, rather than a diagnostic test, and does not replace the diagnostic accuracy of CVS and amniocentesis. Third, there is lack of outcome data for low-risk populations and multiple pregnancies, and it should not yet be offered to women in these populations. Fourth, cell free DNA results cannot be obtained in up to 5% of patients. Fifth, this testing is very expensive compared with other screening options [5].

Larion et al. [23] reported their experience that the introduction of NIPT was significantly associated with a subsequent decrease in CVS and amniocentesis. In the near future, we anticipate that, with the improving technology and lower cost, NIPT will be utilized more frequently, leading to a more reserved use of invasive procedures.

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