

—Mini Review—

Prenatal Genetic Diagnosis through Chorionic Villus Sampling

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Abstract: *Chorionic villus sampling (CVS) is a technique for prenatal diagnosis of the fetal karyotype through cytogenetic analysis, and of Mendelian inherited diseases through molecular or biochemical analysis. Because the sampling technique can be performed in the first-trimester of pregnancy and diagnostic results can be obtained earlier than with amniocentesis, it has been utilized by clinicians in Europe and the US since 1982. CVS includes two methods: a transcervical approach and a transabdominal approach. Both are performed in the 10th week of pregnancy under careful ultrasound guidance to prevent adverse effects, but in comparison with amniocentesis, a slightly higher risk of pregnancy loss has been reported. Moreover, diagnostic accuracy is often disturbed by maternal cell contamination and chromosomal mosaicism of the placenta. Therefore, clinicians must give patients sufficient information on such technical and diagnostic trouble through counseling in order to obtain fully informed consent.*

Key words: *Prenatal diagnosis, Ultrasound guidance, Pregnancy loss, Limb abnormality, Genetic counseling*

Twenty-five years ago, the possibility of sampling tissue from the growing chorion was beyond imagination, but approximately 30 years ago in China, investigators developed a technique for aspiration of chorionic villi to determine fetal sex. Chorionic villus sampling (CVS), which is the method currently utilized, was firstly reported by Old *et al.* [1]. They placed a 1.5 mm plastic catheter transcervically in the villous chorion (chorion frondosum) under ultrasonographic guidance. Thereafter, they reported a 90% sampling success rate in a larger case series [2]. Since then, CVS has been utilized for prenatal diagnosis of some Mendelian

inherited diseases, such as sickle-cell anemia [3], Tay-Sachs disease [4, 5], Duchenne muscular dystrophy [6], and argininosuccinic aciduria [7], and simultaneously for fetal karyotype determination [8, 9].

Prenatal diagnosis through CVS rapidly became widespread after the success of Ward's group. One reason for such widespread use was that the reported accuracy and safety were satisfactory for investigators and parents. Furthermore, the most important reason was that genetic amniocentesis performed in the second trimester of pregnancy has technical limitations; in the use of amniocentesis, the results of prenatal diagnosis of many diseases and karyotyping are not made available until after the 16th week of gestation, because amniocytes must be cultured. Parents who undergo prenatal diagnosis will likely wish to abort an abnormal fetus in early pregnancy as soon as possible after making that decision. In addition, pregnancy termination in the second trimester entails more clinical risk than artificial abortion in the first trimester. The major advantage of CVS over amniocentesis is that the procedure is undertaken in the first trimester of pregnancy, and allows the results of prenatal diagnosis to be available several weeks earlier than with amniocentesis.

The first report of CVS employed the transcervical procedure, in which a sampling tube is inserted through the uterine cervix. Two years after that report, a transabdominal procedure was reported as an alternative method [10]. This method uses a needle for retrieval of chorionic villi through the abdominal and uterine walls under ultrasonographic guidance. Although the amount of villi collected in the transabdominal procedure is less than in the transcervical approach, in our experience almost all genetic or cytogenetic analyses are still possible.

Received: November 17, 2003

Accepted: February 25, 2004

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Method of CVS

The chorion begins to form the villous chorion (chorion frondosum), which will differentiate to become the placenta, at 9–12 weeks gestation. The chorionic tissue contains proliferating villus cells which invade the decidua basalis. CVS is a technique to obtain tissues from the villous chorion. On the side facing the uterine cavity, the villi are compressed with the decidua capsularis as the chorionic sac grows, reducing the blood supply to the villi. Thereafter these villi soon degenerate and make an avascular membrane, the smooth chorion (chorion leave).

Tissues obtained after CVS are often contaminated by decidual components, which originate in the endometrium. Since such maternal tissue contamination results in incorrect analyses, pure villous tissues must be selected under a dissecting microscope. Villous tissues have characteristic morphological features: they show a branching appearance with small buds, and fetal capillaries are visible in those branches (Fig. 1). The villous tissues consist of syncytiotrophoblasts that form an outer layer, and cytotrophoblasts that line the syncytiotrophoblast layer. Rapid direct cytogenetic study is possible with cytotrophoblasts, which are mitotically active.

CVS includes two methods: the transcervical approach and the transabdominal approach. Both are invasive procedures that might cause pregnancy loss. Therefore, patients must be given sufficient information concerning the procedural methods, adverse effects and diagnostic accuracy through counseling. CVS is contraindicated if a patient is suffering from threatened abortion, the symptoms of which are genital bleeding or uterine hardness.

Transcervical sampling (Fig. 2A)

This procedure is performed from the 9th to the 11th week of pregnancy. Ultrasonographic examination before sampling is very important. The uterine position, which is either anterior or posterior flexion, must be recognized in order to insert a catheter in the correct direction. The site and area of the villous chorion, which is observed as a homogeneously hyperechoic area, must also be confirmed. After preprocedural ultrasonography, the patient is placed in the lithotomy position, a speculum is inserted into the vagina, and the vagina and cervix are aseptically prepared with a povidone-iodine solution. The uterine position and the site of the villous chorion are again observed by ultrasound. According to the ultrasound findings, the tip

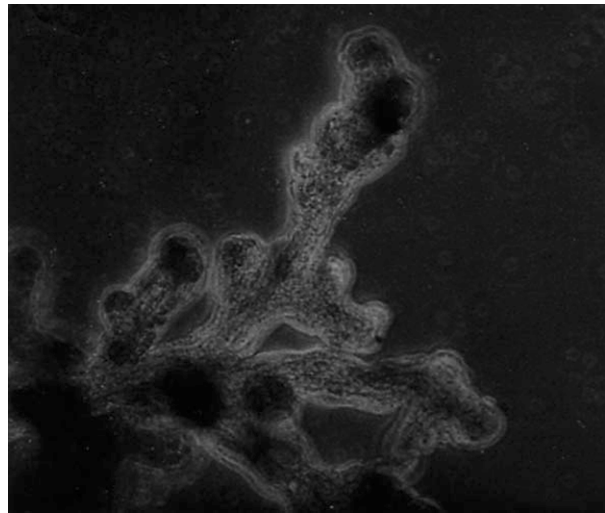


Fig. 1. Chorionic villi observed under a dissecting microscope. Villi have a fibrous structure with branching buds. Fetal capillaries are visible in the structure.

of a plastic catheter (1.5 mm diameter) is bent to pass easily in the direction of the villous chorion. Under continuous ultrasound observation, the catheter is passed through the cervix into the uterine cavity. When the tip of the catheter is difficult to insert in the direction of the villous chorion, a tenaculum, which grasps the anterior lip of the cervix, will help the procedure. The catheter is further inserted parallel to the villous chorion until the catheter tip is located sufficiently in the villous chorion. The obturator is then removed and a 10 ml syringe is connected to the end of the catheter. With gentle negative pressure from the syringe, the catheter is slowly withdrawn. The catheter and the syringe are washed twice with 5 ml of heparinized saline (0.9%, disinfected) in a sterilized tube, and then the saline is poured into a petri-dish. In the case of sampling success, tissue fragments are observed in blood contaminated saline under a dissecting microscope. If there are no villous tissues, the procedure is repeated. More than three repeats must be avoided because of increasing risk of fetal loss.

Transabdominal sampling (Fig. 2B)

The transcervical approach is difficult in some women with a markedly retroverted uterus. In such women, the transabdominal approach is an appropriate alternative method. Moreover, some practitioners who are experienced with amniocentesis prefer the transabdominal approach to the transcervical approach.

The transabdominal approach is difficult when the

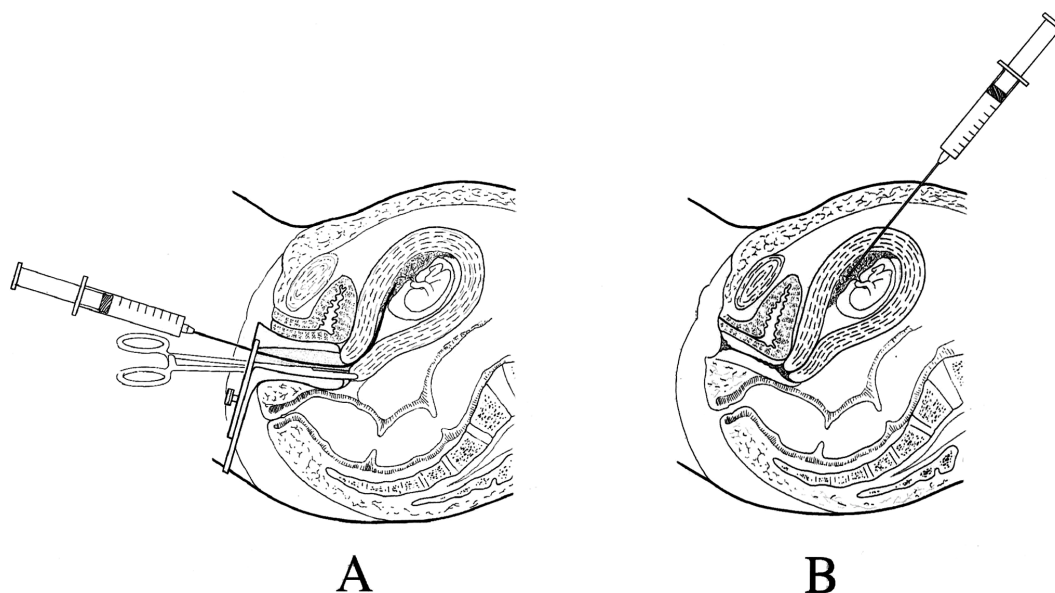


Fig. 2. Schematic representations of CVS techniques. A: transcervical CVS, B: transabdominal CVS. (Reproduced from Silverman and Wapner [24])

villous chorion is located on the posterior wall of the uterus. In such cases, the transcervical approach must be adopted. Therefore, ultrasonographic observation is necessary to confirm the location of the villous chorion. After the ultrasound evaluation, the abdominal skin is disinfected. An 18- or 20-gauge PTC needle is passed through the abdominal and uterine walls in the direction of the villous chorion after local skin anesthesia. The needle must be inserted parallel to the axis of the villous chorion. When the needle tip is located in the center of the villous chorion, the inner needle is removed and a 10 ml-syringe is connected. The outer needle is gently pulled and pushed three times in the villous chorion under continuous negative pressure from the syringe. Then, while maintaining negative pressure, the needle is removed from the abdominal cavity. The needle and syringe are washed with heparinized saline (0.9%, disinfected) in a sterile tube, and the saline is poured in a petri-dish for microscopic observation. Another method for transabdominal sampling that uses a 20-gauge spinal needle has been described [11]. This method seems to be quicker, less uncomfortable, and capable of obtaining a sufficient volume of villous sample.

In contrast to transcervical sampling, which is performed only in the 9th to 11th weeks of pregnancy, transabdominal sampling can be used for placental biopsy even in the second trimester of pregnancy. The basic procedure is the same as described above.

Risks of CVS Procedure

Pregnancy loss is the most serious risk in the CVS procedure. Several large studies in the U.S. and Canada have evaluated the risk of pregnancy loss after the procedure. These studies demonstrated an excess pregnancy loss rate of 0.8% [12] and 0.6% [13] for CVS over amniocentesis, but this difference was not statistically significant. In addition, the Canadian group reported that no significant differences were calculated in the incidences of preterm delivery and low birth weight outcomes between CVS and amniocentesis. In contrast, the MRC European Study [14] showed that 4.6% fewer women in the CVS group had a surviving infant than in the amniocentesis group because of more fetal losses before the 28th gestational week. In these reports, it is suggested that the incidence of pregnancy loss after the CVS procedure is slightly higher than after amniocentesis. Based on our experiences of pregnancy loss after CVS, we inform patients that the incidence of pregnancy loss after CVS is 1.5%, which is relatively higher than after amniocentesis (0.2%). But there have been many reports that pregnancy loss rates increase with the number of aspirations required to obtain a sufficient amount of villous sample [15–18].

Although risk assessment reports of the transabdominal CVS procedure demonstrated that pregnancy loss rates after the procedure were slightly higher than with the transcervical procedure (total

pregnancy loss rate of 2.6% in the transabdominal group vs. 1.8% in the transcervical group), they found no significant difference between the two CVS methods [19–21].

Most women who undergo CVS have no problem after the procedure, but one possible complication is bleeding or spotting from the vagina. These complaints are somewhat more common in women who have had transcervical sampling. Such symptoms may resolve within a day and usually do not present a problem. Moreover, subchorionic hematoma formation, which also does not result in serious problems, is detected in a few women after transcervical CVS and usually disappears within a month. Although there is the possibility of chorioamnionitis after transcervical CVS, the incidence has been reported to be 0.3% before the 20th week of pregnancy [22]. Chorioamnionitis might induce delayed rupture of the membranes.

Most reports have shown no increased risk of fetal structural abnormalities after CVS, but it has been reported that CVS carried out before the 9th week of pregnancy may induce limb abnormalities (oromandibular-limb hypogenesis and terminal transverse limb reduction defect) [23]. Silverman & Wapner [24] suggested that fetal vascular damage, which is associated with cocaine abuse, induces reduction of the fetal peripheral blood circulation, and results in such fetal malformations. In contrast, Jackson *et al.* [25] reported that there were no such malformations in 10,000 cases performed beyond the 10th week of pregnancy. Therefore, in order to prevent fetal limb abnormalities, it is recommended that CVS be performed after the 10th week of pregnancy.

Accuracy of Genetic Diagnosis through CVS

There are several factors that may affect the accuracy of genetic diagnosis with chorionic villi; the primary one is maternal tissue (decidua) contamination, and the second is placental mosaicism. Decidual contamination results in serious diagnostic errors. This kind of contamination can be prevented by the practitioner's experience level and careful tissue selection under a dissecting microscope. By gaining experience and making careful observations with ultrasonography, practitioners are able to prevent decidual contamination.

In the prenatal diagnosis of fetal chromosomes, placental mosaicism can cause problems. Chromosomal mosaicism has been reported in approximately 1% of CVS cases. In genetic counseling

of fetal mosaicism, follow-up analysis with amniocentesis is recommended to confirm whether the mosaicism appears in fetal somatic cells. In approximately 70% of cases the mosaicism is limited to the placenta [12, 26]. Even in fetuses with confirmed placental mosaicism, increased incidences of perinatal complications, such as intrauterine growth retardation and pregnancy loss, have been reported [27, 28].

CVS in Japan

CVS is not a popular technique in Japan. The number of CVS procedures performed was less than 100 per year from 1998 to 2000. Most of those were carried out for fetal cytogenetic analyses, though some were used additionally for biochemical or molecular analyses of hereditary diseases, such as Duchenne muscular dystrophy, congenital adrenal hyperplasia, Tay-Sachs disease, hyperglycinemia, ADA deficiency, and so on (not published in English, the data were prepared by Dr. Sago, National Okura Hospital, for a study supported by the Ministry of Public Health and Welfare). Since clinicians prefer amniocentesis to CVS due to the technical difficulty and higher risk of pregnancy loss in CVS, most fetal chromosome examinations are undertaken with amniocentesis in Japan. Moreover, there are only a few physicians who have experience with CVS. For these reasons, the number of CVS procedures is unlikely to increase in Japan in the future.

Conclusions

The CVS technique is one method for prenatal diagnosis of the chromosomes and Mendelian inherited diseases. Chorionic villi can be subjected to cytogenetic and molecular or biochemical analyses, but in comparison with amniocentesis, a slightly higher risk of pregnancy loss has been reported with CVS. Therefore, providing sufficient information to patient couples is necessary, and fully informed consent must be obtained from the couples in counseling. Practitioners must perform the procedure with careful ultrasound observation, and take care to minimize damage to the villous chorion.

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