62 Chorionic villus sampling

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INTRODUCTION

Invasive prenatal diagnosis methods have gradually seen their importance grow to advances in embryo–fetal medicine, ultrasound imaging, cytogenetic analysis, and molecular biology. Such progress has deeply impacted reproduction behavior for couples at high risk for genetic pathologies.

Information from such methods can reassure couples carrying a pregnancy or, in cases of a positive diagnosis, put the couple in a position to achieve the most appropriate decision, including voluntary pregnancy interruption, fetal therapies, delivery timing and method, and postnatal interventions.

The most widespread invasive techniques employed in prenatal diagnosis centers are chorionic villus sampling (CVS), amniocentesis, and cordocentesis. Each of the techniques has its own advantages and disadvantages, including a miscarriage risk. This risk is related to operator expertise, the method itself, the equipment employed, the sampled quantity, the gestational age at sampling, and maternal age (1–5).

HISTORICAL ASPECTS OF CVS

Research studies of the 1960s and 1970s showed that the chorion could be biopsied through a catheter and that enough placental cells could be obtained so as to allow specific genetic analyses to be done earlier in pregnancy than through amniocentesis. In 1968, in Scandinavia, Mohr made the first attempts of early antenatal genetic diagnosis using sampled chorionic villi (6). He performed a transcervical biopsy of the chorion under direct endoscopic visualization using a straight 6-mm diameter fiber optic device and a sharp blade. The sample was often mixed with amniotic membranes and blood, but it was suitable for chromosomal analysis. Despite his report of a 96% success rate in obtaining chorionic material, this approach was abandoned because of an unacceptably high miscarriage rate and the difficulty in growing chorionic villi cells. Later, fetal chromosome analysis from transcervical placental biopsy prior to termination in early pregnancies was reported by Kullander and Sandahl in 1973 and Hahnemann in 1974 (7,8). In 1975, researchers at the Tietung Hospital in Anshan, China, announced the first successful prenatal diagnostic use of CVS. In an essentially blind approach, the CVS was performed through transcervical aspiration using a 3-mm metal cannula. After its insertion, when soft resistance was encountered, a smaller internal tube was further advanced, and placental material was acquired by syringe suction. Fetal sex was established from chorionic villi cells in early pregnancy according to the presence or absence of sex chromatin (the Barr body) for the purpose of sex preselection. Prenatal losses and spontaneous abortion occurred in 4% to 5% of the cases (9).

Many researchers attempted to sample chorionic villi under endoscopic visualization for first-trimester antenatal

diagnosis, but it was the arrival of real-time ultrasound and the advancement in molecular genetics that rekindled the desire for earlier and safer antenatal diagnosis of genetic diseases.

In 1980, for the first time reported in the literature, Kazy et al. described using ultrasound guidance during chorion sampling (10). Adopting either an endoscopic or an ultrasound-guided approach, they performed fetal sexing and biochemical analyses on chorion tissue biopsied at 6 to 12 weeks of gestation. Instead of cannulas, they used biopsy forceps.

Improved methods for culturing fibroblasts from trophoblast villi were reported by Niazi et al. in 1981 at the St. Mary's Hospital, London (11).

Old et al., from Great Britain, announced using real-time ultrasound and a 1.5-mm biopsy instrument for CVS for diagnosing hemoglobinopathies through molecular methods (12).

In 1983, Ward in London carried out transcervical chorionic sampling with ultrasonic guidance applying a 1.5-mm soft polyethylene catheter and syringe suction (13). His group declared a sampling success rate of 67%, including seven patients for hemoglobinopathy diagnosis through molecular methods.

The success rate of obtaining chorionic material rose from 75% to 96% under ultrasonic guidance, as was demonstrated by Brambati et al. in Milan in 1983. Brambati used a 1.5-mm polyethylene tube with a soft, stainless-steel, malleable obturator inserted into the 1-mm internal barrel. For many years, this device has been the most common design in use (14,15).

Other researchers, such as Dumez in Paris, used a 2-mm rigid biopsy forceps (16).

In 1983, Simoni et al. in Milan described a technique from first-trimester chorionic villi that could be used for direct chromosome preparation and karyotyping as well as for enzyme analyses without needing preliminary culture (17). Chorionic villi were obtained by aspiration or through biopsy from the chorion frondosum, at the edge of the placental disk. With the technique they proposed, in the freshly sampled villus, the inner cytotrophoblast layer (containing actively mitotic cells) could be separated from the outer syncytiotrophoblast cells. All analyses could be performed from these fresh whole villi.

In 1984, transabdominal fine-needle villus aspiration under ultrasound guidance was introduced by Smidt-Jensen and Hahnemann in Copenhagen (18). They registered considerable success in acquiring samples at the first attempt, thus preventing further infective complications and pregnancy loss.

CVS has achieved significant improvements since then, including perfecting the instruments so that they are relatively

easy to manipulate. In addition to advancements in sample acquisition, laboratory techniques have evolved, including those for direct karyotyping, tissue culture, and molecular and biochemical assays of chorionic villi.

The transabdominal approach instantly proved easy to learn and relatively safe. While it registered a higher detection rate, it also guaranteed comfort and privacy to the women undergoing the procedure. It could be practiced at any gestational age, thus spreading quickly in many centers and becoming today the most popular approach of chorion sampling. One particular advantage of CVS is the possibility of performing the procedure very early in pregnancy, starting from the 6th week (although it is generally recommended from the 10th to 12th week). In this way, it is a method that is highly accepted by couples and one apt to diminish physical and psychologic issues related to late diagnosis and eventual voluntary pregnancy interruption due to genetic diseases (19).

INDICATIONS

CVS was initially applied for fetal sexing and prenatal diagnosis of hemoglobinopathies by DNA analysis. However, since then, cytogenetics and DNA analysis techniques have shown serious advances and broadened the number and types of genetic conditions detectable in the antenatal period. As a consequence, the demand for CVS in first-trimester prenatal diagnosis is accordingly making a steady increase.

At present, the primary indications of CVS are chromosomal studies, DNA analysis of genetic disorders, and the prenatal diagnosis of inborn errors of metabolism (Table 1).

Regarding chromosomal studies, CVS is primarily indicated for advanced maternal age (35 years or more), a previous pregnancy with a chromosomal abnormality, a previous pregnancy with multiple anomalies, X-linked diseases, parents with balanced chromosome rearrangements (translocations and inversions), parents with aneuploidies, a past history of recurrent miscarriages, an abnormal ultrasound scan, and oligo-anhydramnios.

It should be noted that the demand for CVS is greater than ever now that first-trimester screening methods for the detection of fetal chromosomal anomalies are developing. As a matter of fact, the risk for fetal aneuploidies related to advanced maternal age remains a common indication for CVS, but the CVS indication has expanded to be employed for

quickly confirming an abnormal karyotype in the first-trimester fetus if a chromosomal abnormality has been suggested by ultrasound and/or biochemical screening. In the last 20 years, the screening of women for fetal Down syndrome has progressed to the evolution of numerous proficient and complex screening strategies that provide the advantages of allowing both superior detection and lower false-positive rates at earlier gestational ages. First-trimester risk assessment of chromosomal defects by the ultrasound measurement of fetal nuchal translucency and the evaluation of the nasal bone (Fig. 1), combined with a biochemical test (pregnancy-associated plasma protein-A, free beta human chorionic gonadotropin), reach a very high detection rate at more than 90% (20–26). Therefore, the choice that patients make should be based on the risk that screening tests generate and not on their age alone (27,28).

In a report in January 2007, “Screening for chromosomal abnormalities,” the American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin advised new guidelines that seek to offer risk evaluation and invasive genetic diagnosis of fetal karyotype to all pregnant women requiring prenatal care before 20 weeks of gestation, regardless of maternal age (29). Among such less common indications for fetal karyotyping are maternal anxiety, polyabortivity, and assisted reproductive techniques by intracytoplasmic sperm injection (30).

The DNA-based diagnoses of single-gene disorders such as cystic fibrosis, hemophilia, muscular dystrophy, and hemoglobinopathies form a group that is expanding with the advance of technology and knowledge of the human genome. About 1% of live births is affected by single-gene disorders, which may be autosomal recessive, autosomal dominant, or X-linked recessive. They imply a high risk of recurrence and are often inadequately treated so that prenatal diagnosis with termination of affected pregnancies is a significant option for couples at risk.

The purpose for prenatal diagnosis of genetic disorders is to determine the carrier status in order to help with genetic counseling of at-risk couples. Most carrier recognition and genetic counseling are retrospective, that is, after the birth of an affected child. Nonetheless, several programs related to

Table 1 Main Indications for Chorionic Villus Sampling
Advanced maternal age
Positive ultrasound and biochemistry first-trimester aneuploidy screening
Parents with chromosomal anomalies
Previous child with chromosomal anomaly
Previous child with plurimalformations
High risk of single-gene disorders
Oligo/anhydramnios
Fetal anomalies detected by ultrasound
X-linked diseases
Inborn errors of metabolism
Infectious diseases

Figure 1 Fetal facial profile at 11 weeks: absent nasal bone.

carrier screening have been launched for populations known to be at risk for specific disorders, as is the case of beta-thalassemia in the Mediterranean area. Beta-thalassemia is the most common single-gene disorder among Italians, where 13% of the people on the Sardinia Island are healthy carriers. Ongoing programs of screening have proved quite effective, and they have resulted in a noticeable decline in the incidence of thalassemia major. Screening programs for Sardinians are based on carrier detection by employing hematologic methods, and genetic diagnoses can be made by identifying specific mutations through DNA analysis in carrier couples. In this way, prenatal diagnosis can be accomplished by mutation analysis on PCR-amplified DNA from chorionic villi (31). Because of advances in molecular techniques, the number of single-gene disorders offered for wide-based population screening will undoubtedly grow.

Ultrasound screening of Down syndrome in the first trimester of gestation may sometimes reveal several fetal abnormalities. Holoprosencephaly, omphalocele, cystic hygroma, diaphragmatic hernia, and megacystis are widely recognized features either of aneuploidies or of certain syndromes. When these are detected in the first trimester, taking advantage of CVS can prompt fetal karyotyping or definitive DNA studies (32).

The inborn errors of metabolism form a vast group of disorders that are individually rare, yet all together embody a major source of human disease and suffering. The majority of these are caused by defects in single genes responsible for encoding enzymes that facilitate conversion of various substrates into different products. Usually, the basis of the problems is the accumulation of substances that are noxious or hamper the normal function as well as the effects of inability to synthesize essential compounds. The basic biochemical defect has been so far identified in more than 300 different inborn errors of metabolism. The greater part of these conditions are indeed rare and new prenatal diagnosis methods for specific disorders are continually being presented; therefore, it is recommended to check with a specialist referral center on the current availability and preferred prenatal diagnosis method. Usually, family history of the disorder with a well-established primary diagnosis in the index case is the indication for prenatal diagnosis but in some cases ultrasound recognizable fetal features may also prompt the prenatal diagnosis.

Sometimes, the chorion tissue must undergo various preparations because more than one analysis is required. Taking into account that chromosome abnormalities are discovered in approximately 1 in 156 live births, and in about 50% of first-trimester spontaneously aborted fetuses, fetal chromosome investigations are an important matter. However, there are cases in which the villi are sampled for other indications (33). As a matter of fact, fetal karyotyping is quite often necessary in addition to the most significant test, even in the absence of specific indications for chromosomal analysis.

Fetal HLA typing could likewise be done in pregnancies of couples at high risk of diseases amenable of treatment by stem cell transplantation, as with beta-thalassemia. In some cases, an alternative to pregnancy termination in the case of an affected fetus could be the prospect of bone marrow

transplantation with an HLA well-matched sibling (34). By CVS, certain congenital infections can be detected such as rubella, toxoplasmosis, cytomegalovirus, and parvovirus (35–37).

Practicing CVS by any means should be a choice contingent on the possible benefits overcoming risks linked to the procedure.

COUNSELING

Before any CVS procedure, adequate genetic counseling is mandatory in order to inform the couple about prognosis, treatment, possible outcomes, complications, and recurrence risks associated with the genetic disease under investigation. In particular, the geneticist or obstetrician should detail advantages and disadvantages in terms of risks associated with the procedure itself, with mention of the abortivity measured in the prenatal diagnosis center providing the care and the one reported in the literature. Information should be included on diagnostic accuracy (i.e., false positives, false negatives, and misdiagnoses), timeline of diagnostic response, gestational age at sampling, success and fail rate of sampling, and all other possible complications. Consent, preferably written, will be given by a woman only after comprehensive, informative, and nondirective counseling.

SAMPLING PROCEDURE

Performing CVS must always be under direct ultrasound visualization through the vagina and the cervix (transcervical, TC-CVS), through the vagina only (transvaginal, TV-CVS), or through the maternal abdomen (transabdominal, TA-CVS) (Table 2).

Transcervical Route

A Chinese group reported success of fetal sexing achieved by transcervical withdrawal of chorionic villi, and this encouraged the development of a number catheters or cannulas for transcervical insertion, all different in material (silver or polypropylene), flexibility, and handling. The instruments employed have gone through variations and there have been alternative methods of obtaining villi; currently, the most common are under transabdominal ultrasound guidance by means of aspiration, by either a cannula or a catheter, and by means of rigid biopsy forceps (38).

For the transcervical procedure, the woman is set in the lithotomy position with comfortable leg supports. The woman’s vulva, perineum, and vagina are cleansed with an antiseptic solution. A sterile speculum (or valve) is inserted into the vagina and opened, and the cervix is swabbed with the same solution. With the speculum in situ, the course of the cervical canal and a more detailed location in the sagittal and transverse view of the uterus is achieved. During the procedure, the assistant (or the operator himself) takes care

Table 2 Chorionic Sampling Approach Under Ultrasound Guidance

Transcervical Chorionic Villus Sampling	TC-CVS
Transvaginal Chorionic Villus Sampling	TV-CVS
Transabdominal Chorionic Villus Sampling	TA-CVS

Figure 2 Transcervical chorionic villi sampling.

Figure 3 Chorionic villi sampling by rigid forceps.

to establish the placental site and its relation to the cervical canal, so as to guide the instrument toward the site of sampling. Sometimes it is necessary to use a tenaculum or a vulsellum for steadying the cervix and passing through the cervical canal.

The aspiration technique represents maybe the most common method of TC-CVS (Fig. 2). It is carried out using a 1.5-mm (16-gauge) cannula of polyethylene with a 1-mm malleable stainless-steel or malleable stylet. To facilitate the transcervical passage into the uterus, the distal 3 to 5 cm of the cannula is slightly curved. The cannula must be passed through the cervix without touching the vaginal walls, and sometimes it is required to steady the cervix until any loss of resistance is perceived at the internal os. The cannula tip is visualized at this point by real-time ultrasound and is advanced under direct visualization into the chorion frondosum. The cannula is positioned parallel to the axis of the developing placenta and advanced almost to its distal end. The metallic obturator (stylet) is then removed, and a 20-ml syringe containing 5 ml of growth medium with heparin is attached to the end of the cannula. Chorionic villi are finally aspirated by applying negative pressure with the syringe while moving the catheter several times through the placenta in the longitudinal axis. The cannula and the attached syringe are then withdrawn. The syringe is to maintain suction throughout sampling and the withdrawal of the catheter to favor villi removal (14).

For sampling with rigid forceps, the woman should be in the same lithotomy position. After thorough local cleaning and accurate ultrasound evaluation, the forceps are introduced under direct transabdominal ultrasound monitoring. They are then gently directed to the sampling site, away from the choriodecidual junctions and margins of the chorionic plate (Fig. 3). The diameter of the forceps is only 2 mm, so care must be taken to constantly visualize the forceps tip, the chorionic plate, and the amniotic sac in order to avoid creating a false passage. Once the site is reached, the sampling itself is performed by opening the jaws and advancing the forceps further into the placenta. They are then closed, and the forceps are slowly withdrawn. Chorion biopsy can be performed using a number of different forceps devices (15). In Europe, a 2-mm straight rigid

forceps (Storz) and a thin-gauged transcervical round-tip steel biopsy forceps with a curved shank (snake forceps, 1.9 mm in diameter, 25 cm in length) are mostly employed.

Transvaginal Route

Transvaginal sampling by a needle guide attached to the vaginal ultrasound probe has also been used. The preparation of the woman is the same as for transcervical sampling. A vaginal 5- to 7.5-MHz ultrasound probe enwrapped in a sterile envelope is inserted into the vagina and connected to a sterile needle guide. Chorionic villi sampling is then accomplished by either a 20-gauge needle with a stylet or a double-needle system, which are transvaginally and transmurally pushed forward into the chorion frondosum and moved to and fro. A target line on the ultrasound monitor sets the puncture depth and ensures reaching the desired biopsy site. Such transvaginal techniques were mostly adopted from vaginosonographically guided puncturing of follicles for oocyte harvesting. They allegedly prove more practical in cases of a fundal placenta with a retroverted uterus; however, this technique is found altogether less advantageous than TA-CVS and transabdominally guided TC-CVS (39).

Transabdominal Route

In the mid-1980s, the transabdominal approach was introduced using either a double- or a single-needle technique, and nowadays, it has become the most widespread technique. The woman should lie on her back on an examining table. The specific area on the woman's abdomen must be adequately cleansed with an aqueous betadine solution (antiseptic). A sterile oil is used so as to allow scanning by the transducer probe, which is commonly wrapped in a sterile drape or glove. The needles and the syringes need to be heparinized prior to skin puncture.

For the double-needle system, special needle guide adapters are necessary. They are available from ultrasound manufacturers and can be coupled with specific probes where the needle passes through a fixed path, either parallel or at an angle to the ultrasonic beam (Fig. 4) (18). First of all, a needle of 1.2 mm outer diameter (18 gauge) is inserted into the needle guide up to the basal plate of the placenta. After that, the stylet

Figure 4 Transabdominal chorionic villi sampling by double-needle system.

Figure 6 Transabdominal chorionic villi sampling by freehand technique. The needle is inserted tangentially to the probe.

Figure 5 Transabdominal chorionic villi sampling by freehand technique. The needle is inserted longitudinally to the probe.

is removed and an aspiration needle of 0.9 or 0.8mm (20 or 21 gauge) is introduced into the first needle and pushed forward into the chorion.

Regarding the single-needle technique, a suitable place of chorion must be identified on the scanner monitor. The puncture is then done freehand by a single 20-gauge spinal needle, inserted tangentially to the greater side of the probe, simultaneously visualizing the needle tip going into the placenta and straight through the shortest route so that only the needle tip is displayed (Fig. 5) (40,41). An oblique insertion, needling at the extremity of the probe, allows observing fully the needle throughout sampling but implies a lengthy course (Fig. 6). Newer needles have a special external coating and emit echo-luminance so as to enhance needle placement.

By adopting the freehand technique, after having reached the sampling site, the needle is connected to a 2.5-mL syringe with a small amount of culture medium or sterile

solution. The displayed needle tip is moved up and down 5 to 10 times in order to favor detachment and suction of villi. All the while, negative pressure must be applied by manual aspiration through the syringe, which is maintained throughout sampling and withdrawing. Some operators use a 30-mL syringe mounted on a holder as an alternative (42).

The need for the transducer probe to be manipulated by the same operator must be emphasized because it results in better hand-eye coordination and the reduction in the number of multiple insertions and the failures associated with the procedure. The single-operator freehand technique necessitates the same operator holding both the probe and the needle during insertion, thus having the sensibility to direct the proper placement of the needle. Sonographic guidance is crucial, but tactile sensation is important as well.

In fact, most procedures require at least three hands. Some operators actually need an assistant to hold the transducer during the connection of the syringe and after withdrawal, while they verify the sampling adequacy and the patient is waiting on the examination table. Other operators hold the transducer in their own hand and ask for assistance for drawing up the tissue.

If obtaining an adequate sample fails, a second insertion is performed. It is important to point out that the procedure is not to be carried out unless the operator is sure of the safe access to the intended chorion site. When difficulties are anticipated, the opinion of a more experienced physician should be sought.

Complications increase significantly if three or more insertions are attempted; therefore, no more than two needle insertions should be performed per session.

According to our own experience, a second attempt to obtain villi is rarely necessary. When the procedure seems too hard and an adequate sample is still not obtained after repeated passes, it may be wise to postpone it to the following days or week. An experienced operator should be consulted for any of these procedures if two attempts at uterine insertion fail to produce an adequate sample for analysis.

CVS Setting

CVS, in any employed route, is generally performed in an outpatient facility or the health-care provider's office. Local anesthesia is not required since it does not deal with sources of pain in the peritoneum and the uterine wall; neither any preoperative nor postoperative medical treatment is usually necessary. Before the CVS procedure, the woman can eat and drink as usual. She may need her partner or another close contact to accompany her to the appointment and support her emotionally or to drive her home afterwards. Based on the clinical experience at our institution, the procedure proves more expeditious if the woman is at ease. The complex range of various psychologic issues related to genetic diagnosis and emotional needs of a woman experiencing CVS requires that the team who performs CVS also develops great sensitivity to the patient's apprehension and anxiety. It appears very helpful to actively involve the woman and maintain a dialogue between her and the operators during the procedure and the fine-tuning of the technical aspects of the CVS, as this minimizes the discomfort and ensures a trusting environment.

During transcervical CVS, the passage of the instrument through the cervix and the suction of villi may prove a cause for cramping, a pinching feeling, and general discomfort (nausea and anxiety). After the sampling, women may experience mild to moderate spotting for the first week.

Regarding TA-CVS, the entire procedure is completed in approximately 10 minutes. Most of the time is usually dedicated to the ultrasonographic investigation and sampling set preparation while the needle insertion itself lasts for a maximum of 10 seconds. On rare occasions, women complain of a rapidly resolving stinging sensation or cramping with the needle penetrating the skin and the uterus. A higher level of pain is likely to be experienced by patients with a thicker abdominal wall (greater than 40mm). At the end of the procedure, all symptoms are resolved. CVS may generate considerable anxiety, but most women find the pain equivalent to that of an intramuscular puncture. A recent study evaluating the woman's experience of pain during TA-CVS reports that the sampling procedure seems most painful at the approach of thicker needles, so the need to use a 19-gauge needle is questioned as acceptable results are obtained with a 20-gauge needle as well (43).

Particular angles of access are also recommended by some experts but the data gathered so far are not exhaustive enough to guide practice. A randomized trial by van Schoubroeck et al. showed that injection of local anesthetic did not reduce pain scores as reported by women undergoing transabdominal punctures (44).

Rhesus status must be considered in every case. According to the guidelines of the ACOG (2001), Rhesus prophylaxis with anti-D immunoglobulin (200–300µg), intramuscularly, should be offered to Rh-negative unsensitized women after each procedure (45,46). However, in cases of being already sensitized, the CVS is contraindicated.

Normally, in a few hours after the sampling, the woman will likely be able to resume normal activities and return home. They are counseled to contact medical staff only if they experience pain, heavy bleeding, or febrile episodes. An ultrasonographic follow-up is recommended when the

diagnostic result is available so as to verify the fetus's well-being and to exclude chorionic hematomas.

MULTIPLE PREGNANCIES

Multiple pregnancies are not, as a general rule an impediment to CVS, but they require a greater amount of expertise and manual skills in order to insure that all fetuses are correctly sampled. Sampling is straightforward when placentas are well separate, but in cases of close proximity, it becomes more complex, as needles need to be serially in all placentas, possibly close to the umbilical cord insertion, in order to insure complete sampling without double passing across the same chorion point. Needles need to be replaced for each sampling (47,48).

In pregnancies with high genetic risk, CVS is better suited than amniocentesis, as an eventual procedure of selective fetus reduction is much simpler and less risky when performed during the first trimester of pregnancy. Careful evaluation and documentation of gestational sacs, fetuses, placentas, and umbilical cords are mandatory when CVS is performed to correctly identify the fetuses when discordant results are obtained and the patient desires selective termination. Sampling error in multiple pregnancies is between 0% and 10% and depends critically on the expertise of the operator. Miscarriage risk is 2% to 4% (49–54).

CONTRAINDICATIONS

Absolute contraindications to TC-CVS are vaginismus and major anomalies of the cervical canal. Cervical polyps and large myomas of the lower uterine segment may represent an important obstacle to the cannula or biopsy forceps. Likewise, a fundal placenta or a posterior placentation in a retroverted uterus may be very difficult to sample by this approach. The transcervical route should be considered a risk of ascending infections in women with active genital infection, even if appropriately treated.

Conversely, it is almost always possible to obtain a sample by the transabdominal route. Actually, there are no valid contraindications to TA-CVS. An appropriate scanner pressure and an accurate bladder filling may favor reaching the sampling site and avoiding intestinal loops in front of the uterus. Myomas may be avoided by real-time guide of needle insertion. In cases of uterine retroflexion, a gentle cervico-vaginal manipulation of the uterus by an assistant may be needed (Fig. 7).

Uterine contractions are occasionally seen, which can interfere with sampling; it is advisable to wait for 10 to 20 minutes for these contractions to cease, even through the help of an antispasmodic drug.

Some contraindications are common for either TC-CVS or TA-CVS.

In any case of active vaginal bleeding, it is advisable to reschedule any sampling procedure to a few days after the bleeding has stopped.

The occurrence of areas of retrochorionic hemorrhage at ultrasonic examination before CVS usually prompts extreme care to avoid it by the cannula or needle.

In cases of preoperative abundant sub-chorionic hemorrhage, deferral is mandatory until after a well-established resolution or to amniocentesis in the second trimester.

Figure 7 Cervico-vaginal manipulation in cases of uterine retroflexion.

Systemic maternal diseases do not represent a contraindication to CVS.

In the presence of inherited bleeding disorders, as in cases of hemophilia carriers with less than 50% factor VIII coagulant activity, prophylactic intravenous infusion of desmopressin (1-deamino-8-D-arginine vasopressin) just before CVS can be offered. This synthetic analog of vasopressin causes an immediate increase in the plasma concentration of factor VIII, von Willebrand factor, and platelet adhesiveness thus preventing abnormal bleeding with few side effects except mild facial flushing and headache (55).

A potential risk exists for maternal–fetal virus transmission as well as patient–clinician transmission in cases of women with blood-borne virus. The fairly small studies available for hepatitis B show no evidence of a transmission risk so that the risk is expected to be very low. The “e” antigen status is likely to be significant for maternal–fetal transmission (56). Although most studies examining human immunodeficiency virus (HIV) suggest that invasive testing may be a risk factor in transmission and recommend avoidance, several authors more recently have suggested that the current risk of HIV vertical transmission associated with early invasive diagnostic techniques is lower than previously reported, provided that antiretroviral therapy is being used (57).

COMPLICATIONS

TA-CVS has a reported abortivity risk around 1%, when performed by an expert hand. Randomized clinical trials have reported a similar fetal loss rate after CVS in the first trimester when compared with amniocentesis at the 16th week. In evaluating the fetal loss rate, spontaneous abortivity in the general population between the 10th and 12th week always needs to be taken into account. Post-procedural complications are highly dependent on the operator’s expertise, gestational age, maternal age, the amount of chorion tissue sampled, the number and duration of needle insertions, bleeding prior to the procedure, and concomitant uterine pathologies.

Minor complications have been reported, like bleeding, spotting, intracervical hematoma, but without sizeable effects on perinatal outcome. In the early 1990s, fetal limb reduction defects have been reported after CVS performed before the

10th week in centers without a proven track record that did not employ standardized techniques. Wider studies on reference centers have however reported rates of limb reduction defects for CVS after the 9th week that are similar to the rates for the general population (5.4/10,000). Accordingly, the WHO-sponsored committee concluded that there is little evidence to suggest a significant risk of congenital defects when CVS is carried out after the 8th completed gestational week and recommended that CVS be performed in referral centers following 9 weeks after the last menstrual period by qualified operators who are adequately trained (58–65).

ANALYSIS

Diagnostic procedure is correlated to the level of expertise of the operator, the cytogeneticist, and the molecular biologist.

The sample of chorionic villus should contain enough material for genetic analysis, generally around 20 to 30 mg. The sample of chorionic tissue should be placed in nutrient medium if cytogenetic analysis has been planned and in a sterile hydro-saline solution for other analyses. The specimen might be mailed to be analyzed but never frozen and should be examined within 24 to 48 hours. Sampled villi need to be examined under an inverted microscope to detect contamination with maternal decidua, which need to be separated so as not to affect diagnosis with unwarranted interpretations.

Two methods are currently available to fully evaluate the fetal karyotype from a CVS specimen: the direct (or short-term) method and the indirect or culture method.

Chorionic villi consist of two cells types: cytotrophoblast cells and mesenchymal core cells. In freshly sampled chorion tissues, the villus inner cytotrophoblast layer beneath the outer syncytiotrophoblast layer contains mitotically active cells, which are rapidly dividing cells, many of which are already in metaphase. These cytotrophoblast actively mitotic cells would provide a reliable means of predicting the fetal karyotype for direct metaphase analysis, without culture. Although the direct analysis of cytotrophoblasts can provide welcome rapid results (24–72 hours), the quality of metaphases obtained is usually less than that obtained by culture.

To enhance the accuracy of CVS results, it is a good practice that villi tissue be analyzed by both the direct (or short-term)

and the indirect (long-term or cultural) method. The first method allows analysis in a few hours, although an incubation of at least 24 hours is recommended with a diagnostic response in 3 to 5 days. The second method analysis is completed after 7 to 10 days.

Currently, confined placental mosaicism is considered the main cause of ambiguous results, which might result in false-positive diagnoses. Following the direct analysis, the majority of discordant findings between chorionic villus samples and the fetus have been false-positive results (1–2%) and only very few have been false negative (1/3000).

With the use of both direct and cultured villus analyses, the incidence of true false-positive results can be greatly reduced (to 1/20,000) and that of true false-negative results can be eliminated. However, when a mosaicism is found after CVS, follow-up amniocentesis and/or cordocentesis is required to clarify results.

Fluorescence in situ hybridization (FISH) can also be performed on noncultured chorion cells following CVS, by using chromosome-specific probes to determine the aneuploidies of chromosomes 13, 18, 21, X, and Y. This has allowed a rapid diagnosis of some selected chromosomal anomalies. It has also enabled the detection of subtle cytogenetic aberrations with a resolution equal to the size of the probe (50–200Kb) that are associated with a clinical phenotype (66,67). Despite the high sensitivity and specificity of interphase FISH for trisomy 13, 18, 21, and sex chromosome aneuploidy (99%), culture failure or noninformative results occur in up to 5.7% of cases and false-positive and false-negative results, although rare, have been reported. For that reason, the American College of Medical Genetics does not recommend therapeutic action on the basis of the results of FISH alone.

An implementation of the chromosomal evaluation in chorion tissue, in the 1990s, was the quantitative fluorescence polymerase chain reaction (QF-PCR), which was derived from the association of techniques of molecular biology to FISH. The technique is based on the amplification of chromosome-specific highly polymorphic DNA short tandem repeats, using fluorescence primers and PCR on chromosomes 13, 18, 21, X, and Y. The QF-PCR, which has comparable sensitivity to the FISH, consents easier identification of maternal contamination and is much less expensive because it is almost entirely automated. QF-PCR is now more common than FISH and many European laboratories consider it the method of choice for rapid aneuploidy testing complementary to full karyotype analysis and, in some specific cases, as an alternative to conventional cytogenetics analysis.

FISH and QF-PCR allow only the detection of aneuploidies involving chromosomes that are specifically tested, usually trisomies of chromosome 13, 18, 21, and sex chromosomes. It means that aneuploidies involving other chromosomes and balanced chromosomal rearrangement can be missed. By using QF-PCR as a stand-alone test, the chances of missing the most common, and the only chromosome anomalies that do not increase in frequency with maternal age, are approximately 1 in 150 abnormal karyotypes, or 1 in 10 to 30,000 samples, based on age distribution; these error rates may be acceptable, although most structural anomalies will be missed (68).

Since chorionic tissue cells provide large sources of DNA suitable for DNA analyses, consequently, CVS is at present the

technique of choice for prenatal diagnosis of genetic diseases with known molecular defects or when a linkage analysis is feasible (i.e., thalassemia, cystic fibrosis, Duchenne muscular dystrophy, hemophilia A, fragile X).

When a specific molecular defect is already known, the research of the defect is performed by different methods using the DNA extracted from villi and amplified by PCR (69).

For DNA-PCR diagnosis of disorders like β -thalassemia, a very small specimen of 1 to 3 mg of chorionic tissue, supplying approximately 1 to 3 μ g of DNA, will suffice, but at least 3 mg of chorionic villi is requested to reduce the risk of co-amplifying the DNA from maternal decidua.

These techniques of PCR-DNA analyses, which enable reliable analysis of a small specimen of villi in 1 to 2 days, are highly efficient and accurate. Misdiagnosis and diagnostic errors are very uncommon (less than 1 in 10,000) in qualified centers when well-defined methods such as variable number tandem repeats are applied to rule out contamination by maternal decidua and blood (31,70).

In utero infections can be documented for agents such as rubella, toxoplasma gondii, cytomegalovirus, varicella zoster, and parvovirus. Diagnosis can be made by extraction of DNA or RNA from chorionic villi and amplification of some well-recognized genes of the suspected infectious agent (35–37).

GUIDELINES

In conclusion, the CVS procedure, when performed by experienced hands, is associated with an approximately 1% to 2% pregnancy loss rate. Adjusting for confounding factors such as gestational age, the procedure-related miscarriage rate following CVS at 10 weeks or later, in very experienced hands, is the same as midtrimester amniocentesis (at best 1 in 300). In less experienced hands, the risk may be higher.

CVS is presently recommended through the transabdominal route after the 9th week of pregnancy with an experienced operator and after genetic counseling. An operator is considered an expert when performing at least 100 samples per year. The training period comprises at least 50 samples under the supervision of a senior tutor. Each operator should undergo yearly audits to verify success rates of samples, fetal loss rate, diagnostic accuracy, and neonatal outcome. Data should be compared with what is reported in literature for reference centers. The analysis laboratory should also ensure an adequate number of exams in order to guarantee the highest degree of certainty (71–73).

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