J. Fetal Med. (June 2017) 4:79–84
DOI 10.1007/s40556-017-0115-5

FETAL PROCEDURES

Chorionic Villus Sampling

Reema Kumar Bhatt[1]

Received: 7 December 2016 / Accepted: 30 January 2017 / Published online: 20 March 2017

Abstract Chorionic villus sampling (CVS) is a form of prenatal diagnosis to determine chromosomal or genetic disorders in the fetus. It entails sampling of the chorionic villus (placental tissue) and testing it for chromosomal abnormalities. It usually takes place at 10–12 weeks' gestation, earlier than amniocentesis or percutaneous umbilical cord blood sampling. CVS was performed for the first time by Italian biologist Giuseppe Simoni. It requires expertise and is safe in the hands of experienced surgeons with a very low fetal loss rate.

Keywords Chorionic villus sampling · Transcervical · Transabdominal · Complications · Oromandibular hypogenesis · Limb-reduction defects · Cytogenetics

Introduction

Chorionic villus sampling (CVS) is a procedure in which small samples of the placenta are obtained for prenatal genetic diagnosis. In experienced hands it is a safe and effective method. It is generally performed in the first trimester, after 10 weeks of gestation. It can be performed using either percutaneous transabdominal (TA) or the transcervical (TC) approach.

✉ Reema Kumar Bhatt
reemakumarbhatt@gmail.com

[1] Department of Obstetrics and Gynecology, Army Hospital Research and Referral, New Delhi 110010, India

Indications

Chorionic villus sampling enables prenatal diagnosis of disorders through cytogenetic, biochemical, or molecular analysis. The most common indications for prenatal genetic diagnosis include the following [1]:

1. Screen positive result (e.g., maternal serum analytes with/without sonographic markers of aneuploidy, cell-free DNA).
2. Congenital anomaly on first trimester ultrasound examination.
3. Female parent is a carrier of a sex-linked disease.
4. Either parent is a carrier of a monogenic (i.e., single gene or Mendelian) disorder, autosomal recessive or dominant.
5. Both parents are carriers of disease.
6. Parent is a carrier of a balanced translocation or other structural chromosome disorder.
7. Previous child with a chromosome abnormality.
8. Maternal age 35 years or older at estimated date of delivery.

Contraindications

1. A relative contraindication to CVS is maternal alloimmunization [2]. If the mother is Rh negative she would require an injection of anti-D after the procedure.
2. Presence of human immunodeficiency virus (HIV), hepatitis B and C in the mother, depending upon the viral load.
3. Cervicovaginal factors (e.g., polyp, myoma, stenosis, vaginismus, infection) which may increase the difficulty of TC-CVS.

Timing

CVS is typically performed between 10 and 14 weeks of gestation. This allows most natural losses because of a chromosomal abnormality etc. Performance very early in pregnancy is associated with an increased risk of limb-reduction defects caused by transient fetal hypoperfusion and vasospastic phenomenon secondary to vascular disruption to the placental circulation. Amniocentesis is preferred at gestations ≥15 weeks because of its, technically, simplicity, patient comfort, and avoids diagnostic uncertainty related to confined placental mosaicism; however, CVS can also be performed after 14 weeks of gestation, usually through a placental biopsy.

Procedure

There is consensus that CVS, both TA and TC, must be performed under continuous ultrasound control. After counseling about the procedure and related complications a written consent is taken. An ultrasound examination should precede the procedure to determine the number of embryos and chorionicity (if twins are present), document fetal viability, localize the placenta, and screen for fetal structural anomalies. A minimum of 30 procedures per year are required to maintain expertise [3]. Technical factors predominantly related to placental location favor one approach over the other. In up to 5% of procedures however operator preference generally guides the decision [4, 5].

Transabdominal CVS

The woman is placed in the supine position and her lower abdomen is prepped with antiseptic solution. TA-CVS procedures is associated with minor pain. There are no studies to prove whether analgesia before CVS reduces pain. However, such studies showed no reduction of pain by prior administration of analgesia or local anesthesia since its use provides dermal but not uterine wall penetration anesthesia. The Cochrane review concluded that in general women who undergo amniocentesis could be informed that pain during the procedure is minor, and that there is currently insufficient evidence to support the use of local anesthesia by rubbing or subfreezing the media [6]. In one randomized control trial "pain scores" were lower in the analgesia group but did not reach statistical significance. The same would apply to CVS.

Techniques for TA-CVS vary significantly both in the size of the needle used (18-gauge, 20-gauge, double needle 17/19-gauge, double needle 18/21-gauge) and method of aspiration (negative pressure by syringe, negative pressure by vacuum aspirator, biopsy forceps). One should use the technique with which one is familiar [7], as there are no published studies comparing clinical outcomes using different techniques. The needle is inserted using either a free-hand technique or a needle-guide attached to the ultrasound probe. The author prefers to use 18-gauge spinal needle (Fig. 1) and free hand technique. The needle is advanced at an angle (Fig. 2a) that allows it to penetrate along the long axis of the placenta (Fig. 2b). The stylet is removed, the medium-containing syringe (20 cc) is mounted on the holder, and the holder is then attached to the hub of the needle. The needle tip is moved back and forth inside the placenta until an adequate sample has been aspirated by the vacuum created in the syringe (Fig. 2c). The sampling system is then withdrawn under negative pressure (Fig. 2d).

Fig. 1 18-Gauge spinal needle

Transcervical CVS (Fig. 3)

The woman is placed in the lithotomy position, the external and internal genitalia are prepped with an antiseptic solution, and a speculum is inserted into the vagina. A single-toothed tenaculum or ring forceps is used to grasp the anterior lip of the cervix and gently pull it toward the operator to bring the uterus into a more axial configuration. Next, under direct TA ultrasound visualization, a metal sound is introduced into the endocervical canal to define its course and curvature. The TC cannula is bent to assume a similar curve and then inserted under ultrasound guidance through the canal and into the placenta. The obturator of the cannula is removed and a 20 mL syringe containing 1 mL of nutrient media is attached to the catheter. Chorionic villi are aspirated as the catheter is moved back and forth inside the placenta several times. After an adequate specimen is obtained, the catheter is withdrawn while keeping the syringe under negative pressure. An alternative TC method uses a biopsy forceps to obtain the placental sample with some added advantage [7, 8]. However the evidence is not strong enough to support change in practice for clinicians familiar with aspiration cannula. Confirmation of cardiac activity at the end of either of the procedure is mandatory.

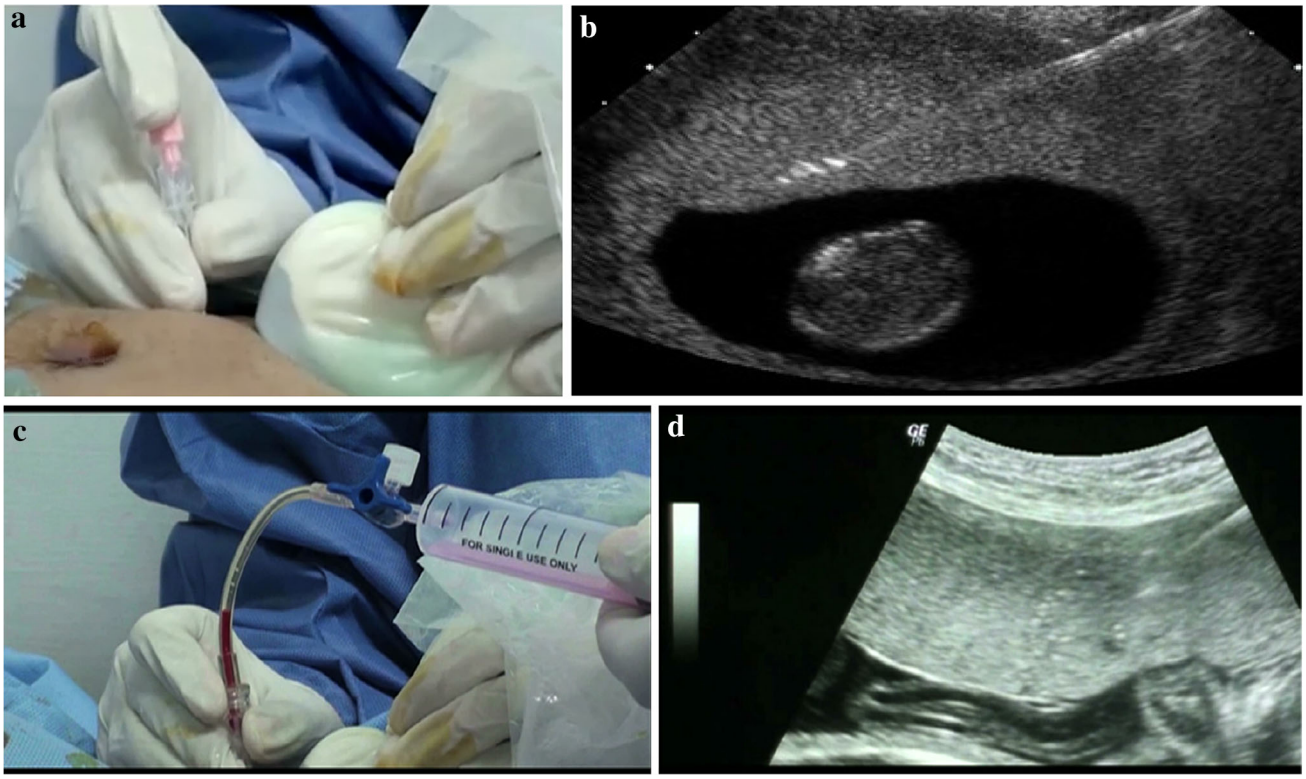

Fig. 2 **a** Needle being inserted for TA-CVS. **b** Needle track in a TA-CVS. **c** Aspiration **d** Withdrawal of needle

The sample material is placed onto a plastic tissue culture dish. Villi appear as white branched structures. At least 5–10 mg of villus tissue is generally required. Blood clots are removed and villi separated from maternal decidua with forceps under a dissecting microscope and the cleaned villi are transferred to an appropriate medium [9].

Postprocedure Care

1. A single intravenous dose of Cefazolin 1 g and 100 mg IM micronized progesterone is administered.
2. Anti-D 300 ug is given if mother is Rh-negative.
3. Activity is restricted for 2 days.
4. Patient is counseled that she might experience mild pain for a couple of days and some spotting is normal, but they should report if there is persistent bleeding, pain, fever, or other concerns.

CVS in Multiple Gestations

Sonographic determination of chorionicity of multiple gestations is essential prior to CVS, as chorionicity determines the number of samples that need to be obtained. In addition, an accurate map of the fetal to placental relationship in multiple gestations is critical for accurate performance of selective termination later in pregnancy. Only a single sample is required in the presence of monochorionic twins. However, there is a possibility of discordant karyotypes in monozygotic twins; therefore, some clinicians sample both fetuses when an anomaly is present. If there are separate placentas, the procedure is similar to CVS for a singleton pregnancy (but with two separate needle insertions). In up to 6% of cases follow-up amniocentesis is required [10, 11].

Evaluation of Sample for Genetic Results

By direct examination of cytotrophoblast (i.e., direct method) rapid karyotyping can be achieved within 2–48 h of sampling since these cells have a high mitotic index and can be examined in metaphase. However, due to the risk of false positive results, long-term (1 week) cultures of mesenchymal cells should be performed concurrently as these cells better reflect fetal, rather than the placental, genotype. Assessment by chromosomal microarray analysis of chorionic villi may also be done [12]. A review of data from several published studies estimated that as far as performance of test is concerned the sensitivity CVS for detection of nonlethal chromosomal aneuploidies was 99.6% (false negative 0.4%)

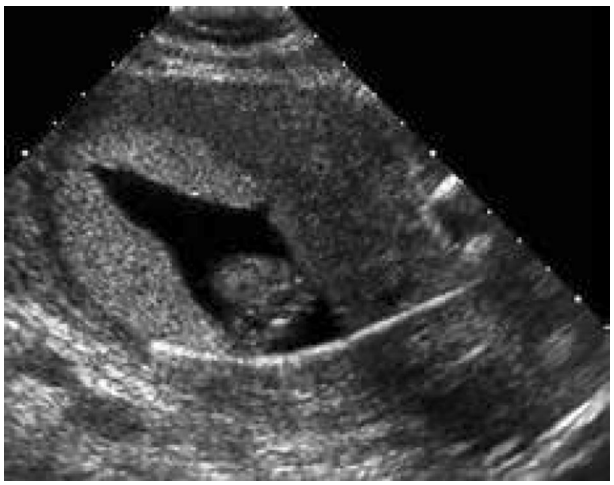

Fig. 3 Transcervical CVS

[13]. The specificity of CVS was 99.90% (false positive 0.1%).

Complications

The most serious complications from CVS are fetal loss and injury to the fetus.

Bleeding

One-third of women may report with spotting after CVS [28], while 7–10% of TC-CVS procedures and less than 6% of TA-CVS procedures may be associated with more persistent bleeding [13].

Infection

Clinically evident infectious complications have been reported rarely [28]. Fetal loss can be caused by clinical or subclinical intrauterine infection. Cervicovaginal flora may contaminate TC catheter and TA catheter can be contaminated with skin flora or puncture of the bowel.

Fetomaternal Hemorrhage

This may result from placental disruption following CVS procedure. The actual amount of bleeding is usually small relative to the total fetoplacental blood volume [29]. Therefore, unsensitized Rh negative women should receive anti-D immunoglobulin following CVS [2].

Rupture of Membranes

Delayed rupture of membranes was reported in 0.3% of cases days to weeks after the procedure [30]. It is rare to have acute rupture of membranes. There is no increase in fetal and infant mortality, prematurity, low birthweight, non-reassuring fetal status, or limb reduction defects associated with CVS as per the population-based cohort study of women 35–49 years of age that compared infant morbidity of those exposed to CVS versus those not exposed reported [31].

Total Fetal Loss Rate

CVS is associated with a higher rate of fetal loss than amniocentesis, as suggested by bulk of evidence from randomized trials [13, 14]. However, it is transcervical (TC)-CVS only and not TA-CVS that appears to be associated with excess risk. A systematic review of 16 cohort studies on complications of CVS calculated total fetal loss rates of 0.7% within 14 days of TA-CVS, 1.3% within 30 days, and 2% for loss anytime during pregnancy [14]. This risk is probably very small as systematic review of studies with unselected control populations reported no significant difference in the rate of miscarriage between CVS and unselected control groups not undergoing an invasive procedure [15] (Table 1). The increasing use of cell-free DNA testing has significantly decreased the rate of invasive testing and the safety/risks of CVS (TA or TC), as reported in literature before 2011, may not be applicable in the current practice [16, 17]. As a result, it is increasingly difficult for operators to learn and maintain

Table 1 Studies reporting on the rate of miscarriage before 24 weeks gestation in women who had chorionic villus sampling (CVS) and those who did not undergo any invasive procedure

References	CVS group		Control group		
	Miscarriage rate		Miscarriage rate		
	GA (weeks)	Total (n)	(n [% (95% CI)])	Total (n)	(n [% (95% CI)])
Lau et al. [32]	12 (10–21)	1355	25 [1.85 (1.20–2.71)]	1125	13 [1.16 (0.62–1.97)]
Odibo et al. [33]	11 (10–14)	5148	138 [2.68 (2.26–3.16)]	4803	161 [3.35 (2.86–3.90)]
Tabor et al. [34]	10 (9–14)	31,355	589 [1.88 (1.73–2.03)]	633,308	25,063 [3.96 (3.91–4.01)]
Akolekar et al. [35]	12 (11–14)	2396	44 [1.84 (1.34–2.46)]	31,460	360 [1.14 (1.03–1.27)]

appropriate technical skills, which may affect procedure-related fetal loss rates [18].

Limb-Reduction Defects and Oromandibular Hypogenesis

The risk falls with advancing gestational age and approaches the background population rate at >11 weeks of gestation [25]. An increased rate of transverse limb abnormalities has been reported when CVS is performed before nine weeks of gestation [26]. Oromandibular hypogenesis may be associated with limb reduction in some cases (called the oromandibular-limb hypogenesis syndrome) [27].

Failure to Obtain a Sample

The sampling success rate in the majority of the studies is at least 99% and is similar for TC-CVS and TA-CVS.

Confined Placental Mosaicism

Confined placental mosaicism refers to a discrepancy between the genotype of the placenta and the genotype of the embryo/fetus. Mosaicism is identified in 1–2% of CVS samples, but confirmed in the fetus in only 11% of these cases [19, 21, 23]. Mosaicism in CVS has both diagnostic and prognostic implications because placental function can be affected, leading to miscarriage, fetal growth restriction, fetal death, or stillbirth [24].

Diagnostic Uncertainty and Misdiagnosis

In one series of over 62,000 procedures, the false negative rate with CVS was extremely low (0.03%) [19]; therefore, patients can be reassured of an unaffected fetus if CVS is normal or the mosaic karyotype is confined to direct preparations (i.e., trophoblastic cells) and the long-term cultures (i.e., mesenchymal cells) have a normal chromosome complement. However, when the mosaic karyotype is found in mesenchymal cells amniocentesis should be performed to rule out a false positive test. If the chorionic villus sample is inadequate for both direct preparations and long-term cultures, long-term culture appears to be more reliable than a direct preparation [20].

The certainty that the established karyotype reflects the fetal genotype is lower with CVS therefore the need for follow-up samples is significantly higher after CVS than after amniocentesis [21].

Maternal Cell Contamination

Maternal cells may occasionally remain and grow in culture. For detection and interpretation of maternal cell contamination in long-term cultures, guidelines have been developed [22]. The operator must learn to separate the villi from maternal decidual tissue.

Conclusion

Chorionic villus sampling is an ambulatory procedure in which small samples of the placenta are obtained for prenatal genetic diagnosis under real-time ultrasound guidance, typically between 10 and 14 weeks of gestation. If direct preparations are performed, preliminary cytogenetic results are available within 48 h and final results (based on long-term culture) are reported in 7–10 days. Compared with mid-trimester amniocentesis, CVS offers greater privacy, a shorter duration of anxiety since results are available earlier in gestation and, if pregnancy termination is performed, termination at an earlier gestational age is more widely available and has lower risks than mid-second trimester termination procedures. However, CVS is associated with a higher rate of diagnostic uncertainty than amniocentesis. The choice of procedure depends upon the woman's personal appraisal of the risks and benefits of each technique.

References

1. Wapner RJ, Toy EC. Chorionic villus sampling. Chapter 24. http://fleischer_ch24%20(5).pdf. Accessed 11 Mar 2017.
2. Moise KJ Jr, Carpenter RF Jr. Increased severity of fetal hemolytic disease with known rhesus alloimmunization after first trimester transcervical chorionic villus biopsy. Fetal Diagn Ther. 1990;5:76–8.
3. Royal College of Obstetricians and Gynaecologists. Amniocentesis and chorionic villus sampling. Green top guideline No. 8, June 2010. http://www.rcog.org.uk/files/rcog-corp/GT8Amniocentesis0111.
4. Jackson LG, Zachary JM, Fowler SE, et al. A randomized comparison of transcervical and transabdominal chorionic-villus sampling. The U.S. National Institute of Child Health and Human Development Chorionic-Villus Sampling and Amniocentesis Study Group. N Engl J Med. 1992;327:594.
5. Silver RK, MacGregor SN, Sholl JS, et al. Initiating a chorionic villus sampling program. Relying on placental location as the primary determinant of the sampling route. J Reprod Med. 1990;35:964.
6. Mujezinovic F, Alfirevic Z. Analgesia for amniocentesis or chorionic villus sampling. Cochrane Database Syst Rev. 2011;(11):CD008580. doi:10.1002/14651858.CD008580.pub2.
7. Young C, von Dadelszen P, Alfirevic Z. Instruments for chorionic villus sampling for prenatal diagnosis. Cochrane Database Syst Rev. 2013;1:CD000114.
8. Von Dadelszen P, Sermer M, Hillier J, et al. A randomised controlled trial of biopsy forceps and cannula aspiration for transcervical chorionic villus sampling. BJOG. 2005;112:559.
9. Brown L, Abigania M, Warburton D, Brown S. Validation of QF-PCR for prenatal aneuploidy screening in the United States. Prenat Diagn. 2006;26:1068.

10. Pergament E, Schulman JD, Copeland K, et al. The risk and efficacy of chorionic villus sampling in multiple gestations. Prenat Diagn. 1992;12:377.
11. Wapner RJ, Johnson A, Davis G, et al. Prenatal diagnosis in twin gestations: a comparison between second-trimester amniocentesis and first-trimester chorionic villus sampling. Obstet Gynecol. 1993;82:49.
12. Breman A, Patel A. Preparation of chorionic villus samples for metaphase chromosome analysis and chromosomal microarray analysis. Curr Protoc Hum Genet. 2012;75:8.3.1–8.3.9.
13. Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. Cochrane Database Syst Rev. 2003;(3):CD003252.
14. Mujezinovic F, Alfirevic Z. Procedure-related complications of amniocentesis and chorionic villous sampling: a systematic review. Obstet Gynecol. 2007;110:687.
15. Akolekar R, Beta J, Picciarelli G, et al. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2015;45:16.
16. Louis-Jacques A, Burans C, Robinson S, et al. Effect of commercial cell-free fetal DNA tests for aneuploidy screening on rates of invasive testing. Obstet Gynecol. 2014;123(Suppl 1):67S.
17. Turner AL, Rad S, Afshar Y, et al. Declining rate of invasive procedures for prenatal diagnosis in the era of noninvasive prenatal testing. Obstet Gynecol. 2014;123(Suppl 1):196S.
18. Caughey AB, Hopkins LM, Norton ME. Chorionic villus sampling compared with amniocentesis and the difference in the rate of pregnancy loss. Obstet Gynecol. 2006;108:612.
19. Hahnemann JM, Vejerslev LO. Accuracy of cytogenetic findings on chorionic villus sampling (CVS)—diagnostic consequences of CVS mosaicism and non-mosaic discrepancy in centres contributing to EUCROMIC 1986–1992. Prenat Diagn. 1997;17:801.
20. van den Berg C, Van Opstal D, Polak-Knook J, Galjaard RJ. (Potential) false-negative diagnoses in chorionic villi and a review of the literature. Prenat Diagn. 2006;26:401.
21. Los FJ, van Den Berg C, Wildschut HI, et al. The diagnostic performance of cytogenetic investigation in amniotic fluid cells and chorionic villi. Prenat Diagn. 2001;21:1150.
22. Nagan N, Faulkner NE, Curtis C, et al. Laboratory guidelines for detection, interpretation, and reporting of maternal cell contamination in prenatal analyses a report of the association for molecular pathology. J Mol Diagn. 2011;13:7.
23. Stetten G, Escallon CS, South ST, et al. Reevaluating confined placental mosaicism. Am J Med Genet A. 2004;131:232.
24. Taylor TH, Gitlin SA, Patrick JL, et al. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. Hum Reprod Update. 2014;20:571.
25. Firth H. Chorion villus sampling and limb deficiency—cause or coincidence? Prenat Diagn. 1997;17:1313.
26. Olney RS, Khoury MJ, Alo CJ, et al. Increased risk for transverse digital deficiency after chorionic villus sampling: results of the United States Multistate Case-Control Study, 1988–1992. Teratology. 1995;51:20.
27. Firth HV, Boyd PA, Chamberlain PF, et al. Analysis of limb reduction defects in babies exposed to chorionic villus sampling. Lancet. 1994;343:1069.
28. Rhoads GG, Jackson LG, Schlesselman SE, et al. The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. N Engl J Med. 1989;320:609.
29. Katiyar R, Kriplani A, Agarwal N, et al. Detection of fetomaternal hemorrhage following chorionic villus sampling by Kleihauer Betke test and rise in maternal serum alpha feto protein. Prenat Diagn. 2007;27:139.
30. Brambati B, Oldrini A, Ferrazzi E, Lanzani A. Chorionic villus sampling: an analysis of the obstetric experience of 1,000 cases. Prenat Diagn. 1987;7:157.
31. Cederholm M, Haglund B, Axelsson O. Infant morbidity following amniocentesis and chorionic villus sampling for prenatal karyotyping. BJOG. 2005;112:394.
32. Lau KT, Leung YT, Fung YT, Chan LW, Sahota DS, Leung NT. Outcome of 1,355 consecutive transabdominal chorionic villus samplings in 1,351 patients. Chin Med J (Engl). 2005;118:1675–81.
33. Odibo AO, Dicke JM, Gray DL, Oberle B, StamilioDM Macones GA, Crane JP. Evaluating the rate and risk factors for fetal loss after chorionic villus sampling. Obstet Gynecol. 2008;112:813–9.
34. Tabor A, Vestergaard CH, Lidegaard O. Fetal loss rate after chorionic villus sampling and amniocentesis: an 11-year National Registry Study. Ultrasound Obstet Gynecol. 2009;34:19–24.
35. Akolekar R, Bower S, Flack N, Bilardo CM, Nicolaides KH. Prediction of miscarriage and stillbirth at 11–13 weeks and the contribution of chorionic villus sampling. Prenat Diagn. 2011;31:38–45.