

Amended Canadian Guideline for Prenatal Diagnosis (2005) Change to 2005-Techniques for Prenatal Diagnosis

The following guidelines for prenatal diagnosis have been amended by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC) and approved by the Executive and Council of the SOGC. These guidelines are an update of the guidelines previously published (Canadian College of Medical Geneticist and Society of Obstetricians and Gynaecologists of Canada, 1993 and 2001) and are to be used in connection with the 2001 version. These guidelines will also be available on the Internet at www.sogc.org and will be updated regularly.

PRINCIPAL AUTHOR

R. D. Wilson, MD, FRSCS, Philadelphia PA

GENETICS COMMITTEE

Greg Davies, MD, FRCSC, Kingston ON

Alain Gagnon, MD, FRCSC, Vancouver BC

Valerie Desilets, MD, FRCSC, Montreal QC

Gregory J. Reid, MD, FRCSC, Winnipeg MB

Anne Summers, MD, FRCSC, Toronto ON

Philip Wyatt, MD, PhD, Toronto ON

Victoria M. Allen, MD, MSc, FRCSC, Halifax NS

Sylvie Langlois, MD, FRCPC, Vancouver BC

The quality of evidence reported in this document has been described using the Evaluation of Evidence criteria outlined in the Report of the Canadian Task Force on the Periodic Health Exam (Table 1).⁷⁰

J Obstet Gynaecol Can 2005;27(11):1048–1054

INTRODUCTION

Invasive prenatal diagnosis techniques include chorionic villus sampling (CVS), amniocentesis, cordocentesis or percutaneous umbilical blood sampling (PUBS), fetal tissue

Key words: Prenatal diagnosis, amniocentesis, early amniocentesis, chorionic villus sampling, procedure, benefits, risks

sampling, as well as embryoscopy and fetoscopy (Table 2). Some diagnostic results may be obtained by more than one technique: for example, fetal karyotype can be obtained from cells from amniocentesis, chorionic villus sampling, or fetal blood sampling.

First trimester screening for aneuploidy and congenital anomalies, using ultrasound for fetal nuchal translucency measurements and maternal serum biochemical markers, have been developed with trisomy 21 detection rate of 60 to 90% with a screen positive (false positive) of approximately 5% to 10%.^{1–7} Diagnostic invasive prenatal diagnosis with CVS at 10 to 14 weeks is offered for first trimester positive screening, while first or second trimester positive screening tests may undergo diagnostic invasive prenatal diagnosis with amniocentesis after 15 weeks.

AMNIOCENTESIS

Amniocentesis is an ultrasound-guided invasive prenatal diagnosis procedure usually performed after 15 weeks gestational age for determination of fetal karyotype, molecular, and biochemical abnormalities (Table 2). The 2 most common tests performed on the amniotic fluid are the fetal karyotype from fetal and membrane cells in the amniotic fluid after tissue culturing or direct fluorescent insitu hybridization (FISH) techniques, and direct measurement of amniotic fluid alpha fetoprotein (AFAFP). Other genetic diagnoses can be obtained by biochemical or molecular techniques after discussion with the local prenatal diagnosis centre. Results can generally be obtained prior to 20 weeks gestational age. The fetal karyotype will usually take 1 to 3 weeks from the time of amniocentesis, depending on the cytogenetic laboratory. The major disadvantage of amniocentesis is that results of the prenatal diagnosis are not available until 17 to 20 weeks gestational age. If genetic

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Table 1. Criteria for quality of evidence assessment and classification of recommendations

Level of evidence*	Classification of recommendations†
I: Evidence obtained from at least one properly designed randomized controlled trial.	A. There is good evidence to support the recommendation for use of a diagnostic test, treatment, or intervention.
II-1: Evidence from well-designed controlled trials without randomization.	B. There is fair evidence to support the recommendation for use of a diagnostic test, treatment, or intervention.
II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group.	C. There is insufficient evidence to support the recommendation for use of a diagnostic test, treatment, or intervention.
II-3: Evidence from comparisons between times or places with or without the intervention. Dramatic results from uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category.	D. There is fair evidence not to support the recommendation for a diagnostic test, treatment, or intervention.
III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.	E. There is good evidence not to support the recommendation for use of a diagnostic test, treatment, or intervention.

*The quality of evidence reported in these guidelines has been adapted from the Evaluation of Evidence criteria described in the Canadian Task Force on the Periodic Health Exam.⁷⁰

†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the Canadian Task Force on the Periodic Health Exam.⁷⁰

abnormalities are identified and the patient requests termination of pregnancy, the techniques of pregnancy termination such as induction of labour or dilatation and evacuation (D+E), carry a greater emotional and physical risk to the woman than a first trimester termination by dilatation and curettage (D+C).

PROCEDURE

Ultrasound is performed prior to amniocentesis to determine fetal cardiac activity, fetal gestational age, location of placenta, amniotic fluid volume, number of fetuses, and uterine factors such as fibroids, amnion-chorion separation or contractions. More detailed fetal anatomy may be included, depending on the centre and the age of the pregnancy. The needle insertion site is identified by the ultrasound information regarding fetal position, amniotic fluid volume, and placental location. Avoidance of the placenta is recommended. Although published results regarding transplacental amniocentesis have not shown significant increased risks for miscarriage,⁸ increased risk of fetal-maternal transfusions has been reported.⁹ The concurrent use of ultrasound with amniocentesis is recommended to allow continuous observation of the fetus, amniotic fluid, and position of the needle tip.

Sterile technique, including sterile gloves and a procedure tray with antiseptic solution, gauze pads, forceps, and sterile drape should be used. The skin insertion site is cleaned with an antiseptic solution. The use of local anesthetic in the abdominal wall is not generally necessary. The procedure is

usually performed with a 20- to 22-gauge spinal needle using a single continuous movement of the needle through the abdominal and uterine wall. It is important that entry into the amniotic sac is a sharp thrust to avoid “tenting” of the amnion. A 10 to 20 cc syringe is used to aspirate the amniotic fluid following removal of the needle stylet. The volume of amniotic fluid removed is 15 to 30 cc and depends on the indication for prenatal diagnosis and the gestational age at the time of the procedure. The removal of the spinal needle reverses the technique used for insertion.

Removal of the amniotic fluid generally takes less than 1 minute. The patient may experience some mild uterine cramping and pressure sensation. The amniotic fluid is generally similar in appearance to dilute urine. Occasionally blood-tinged amniotic fluid may be obtained, generally due to maternal bleeding into the amniotic cavity at the time of the procedure. If the patient has previously had a history of antepartum bleeding, the amniotic fluid may be brown or dark red in colour due to blood pigments being absorbed across the chorio-amnionic membranes. The presence of discoloured fluid on amniocentesis is associated with an increased risk of pregnancy loss.¹⁰

No more than 2 uterine needle insertions into or through the uterine wall are recommended. If the procedure is unsuccessful, further attempts can be made with a delay of at least 24 hours.

Freshly blood-stained amniotic fluid should be separately analyzed by a Kleihauer test and cell count to determine whether the new blood is maternal or fetal. If the blood is

Table 2. Summary of Amniocentesis and Chorionic Villus Sampling (CVS) Information

	Amniocentesis	CVS
Procedure	Amniotic fluid removed by needle and syringe	Chorionic villi removed by transcervical (TC) catheter or biopsy forceps (BF) and syringe or transabdominal (TA) needle insertion
Timing	15–17 weeks	TA 10–32 weeks TC 10 ⁺⁰ –11 ⁺⁶ weeks
Added risk of miscarriage due to procedure	0.5%–1.0%	TA 1%–2% TC 2%–6%
Fetal malformation risks	—	1 in 3000 vascular limb malformation (suggested but not proven)
Chance of successful sampling	Approximately 99%	Approximately 99%. If unsuccessful, can follow with amniocentesis
Time required for cytogenetic diagnosis	1–3 weeks (FISH may be available)	2–3 weeks (rapid direct technique may be considered in specific situations)
Accuracy (chromosomes) Aneuploidy and major structural rearrangement	Highly accurate	Highly accurate
Mosaicism	True fetal mosaicism – rare	Confined placental – 1.0%–2.0%
Open neural tube defects (NTDs)	AFP in amniotic fluid detects approximately 95% of NTDs	Other tests required for detection of NTDs

FISH: fluorescence in situ hybridization
AFP: alpha fetoprotein

fetal, the AFAFP value may be elevated without a congenital anomaly as the etiology. Rhesus prophylaxis is given if the woman is known to be Rhesus negative according to SOGC guidelines.¹¹ Patients are generally requested to have limited activity for 12 to 24 hours following the amniocentesis procedure, but the efficacy of decreased activity in reducing the risk of pregnancy loss has not been well studied.

DISADVANTAGES AND RISKS OF AMNIOCENTESIS

A) Fetal Loss

Fetal loss after amniocentesis is estimated to be 1 in every 100 to 600 procedures above the background loss rate.^{12–19}

B) Infection

The risk of infection introduced at the time of the amniocentesis is estimated to be 1 to 2 in 3000 procedures.²⁰ Recent information indicates that approximately 10% to 50% of post-amniocentesis losses have evidence of low-grade infections at the time of the procedure with increased cytokine levels in the amniotic fluid.^{21,22}

C) Fetal Injury

Serious fetal injuries at the time of amniocentesis are rare with or without continuous ultrasound guidance. Small skin dimpling lesions have been reported following contact of the fetus with the needle, but these are generally minimal

and the specific anatomic location may be the only consideration.^{23–25}

D) Other Complications

Complications without fetal loss following amniocentesis include continued leakage of amniotic fluid, bleeding, and uterine irritability. These complications are estimated to occur in 1% to 5% of procedures.^{18,22,27} These complications are generally self-limited. Recommendations may include bedrest, but this has not been well studied, and additional serial ultrasound monitoring if continued amniotic fluid leakage is present. The benefit of antibiotic use with amniotic fluid leakage has not been evaluated. Persistent amniotic fluid leakage associated with ongoing severe oligohydramnios can lead to pulmonary hypoplasia and arthrogryposis in the newborn.

TWIN PREGNANCY

The number of multiple pregnancies is increasing due to advancing maternal age and assisted reproductive technology. Multiple pregnancies have an increased risk for maternal age-specific chromosomal disorders (dizygotic) and fetal anatomical disorders (monochorionic > dizygotic).^{28–30} It is necessary to define chorionicity (monochorionic, dichorionic), placental location, and the presence or absence of separating membranes and their thickness by ultrasound assessment in the first or second trimester. A thorough descriptive localization of twin A or

B (right- or left-sided twin with placental location) is necessary, especially when an abnormality is identified in only 1 twin. Genetic amniocentesis (second trimester) for multiple pregnancies requires that all amniotic sacs are individually sampled. Separate ultrasound-guided needle insertion with or without the use of dye (indigo carmine) is the preferred method to reduce the risk of amniotic fluid contamination but double sac sampling with 1 needle insertion has been reported.^{31–36} Spontaneous pregnancy loss rate before 24 to 28 weeks' (with no invasive procedures) in twin pregnancies is estimated at 3.4 to 5.89%.^{33,37–39} The procedure-related loss rate in twin pregnancies is estimated at 1% to 4%.^{33–36} The procedure risk is estimated to contribute to the fetal loss rate for approximately 5 weeks.³³

EARLY AMNIOCENTESIS

Findings from the large Canadian multicentred prospective randomized trial^{18,40} comparing early amniocentesis (11 to 12 weeks 6 days) and mid-trimester amniocentesis (15 to 16 weeks 6 days) have confirmed the findings from smaller randomized trials. Significant differences for early amniocentesis compared with mid-trimester amniocentesis were found for: (1) total fetal losses including pre-procedure, post-procedure, stillbirth, and neonatal death (7.6% in the early amniocentesis group vs. 5.95% in the mid-trimester amniocentesis group, $P = 0.012$), for newborn clubfoot (1.3%, 0.1%, $P = 0.0001$), and for post-procedural amniotic fluid leakage (3.7% vs. 1.5%, $P = 0.0007$). Cytogenetic culture failures were also more likely in the early amniocentesis group (1.8% for early amniocentesis vs. 0.2% for mid-trimester amniocentesis, $P < 0.0001$), requiring additional invasive prenatal diagnosis techniques for these women if further diagnosis was requested. There was no significant difference in the incidence of neonatal respiratory disease or congenital hip dislocation when comparing the 2 groups. Early amniocentesis does not appear to be appropriate for routine prenatal diagnosis at gestational ages of 11 to 13 weeks 6 days gestation.

A recent randomized trial evaluated the safety and accuracy of amniocentesis and transabdominal chorionic villus sampling (CVS) performed at 11 to 14 weeks of gestation.⁴¹ There were 3775 women randomized into 2 groups (1914 to CVS; 1861 to amniocentesis). The primary outcome measure of a composite of fetal loss plus preterm delivery before 28 weeks of gestation in cytogenetically normal fetuses was similar for both groups (2.1% for CVS vs. 2.3% for amniocentesis, $P = \text{NS}$). Spontaneous pregnancy losses before 20 weeks and procedure-related indicated termination appeared increased in the amniocentesis groups (RR 1.74, 95% CI, 0.94–3.22, $P = .07$). There was a 4.65-fold increase in the rate of talipes equinovarus after early amniocentesis (95% CI, 1.01–21.5, $P = .017$). The study concluded

that amniocentesis at 13 weeks carries a significantly increased risk of talipes equinovarus compared with CVS and a possible increase in early, unintended pregnancy loss.

CHORIONIC VILLUS SAMPLING

Chorionic villus sampling (CVS) is the most common first trimester invasive prenatal diagnosis technique for evaluation of fetal karyotype, molecular, and biochemical abnormalities (Table 2). CVS is an ultrasound-guided technique that is usually performed in the first trimester between 10 and 13 weeks 6 days gestation. Although the procedure was initially developed as a transcervical technique, both transcervical and transabdominal techniques are currently used. In contrast to amniocentesis, which obtains amniotic fluid, the CVS obtains chorionic tissue from the developing placenta.

PROCEDURE

Ultrasound is performed prior to CVS to determine fetal cardiac activity, gestational age, number of fetuses, and uterine factors such as fibroids, amnion-chorion separation or contractions. Concurrent use of ultrasound with CVS is recommended to allow continuous observation of the biopsy forceps, catheter, or needle tip. Sterile technique, including sterile gloves and a procedure tray with antiseptic solution, gauze pads, and sterile speculum should be used.

The transcervical chorionic villus sampling technique uses either a biopsy forceps, or a flexible plastic catheter. Prior to insertion of the transcervical instrument, a speculum is placed in the vagina and the cervix and vaginal walls are cleansed with antiseptic solution. In the majority of cases, further manipulation of the uterus and cervix by a tenaculum is not necessary. Transcervical CVS utilizing the biopsy forceps requires directing the forceps through the cervix and into the placental tissue under continuous ultrasound guidance. A biopsy is performed and the forceps is gently withdrawn. Transcervical CVS utilizing the catheter requires directing the catheter, with a plastic or metal obturator whose shape can be moulded to allow the catheter to pass, attached to a 20- to 30-cc syringe, through the cervix and into the placental tissue under continuous ultrasound guidance. The catheter is withdrawn through the placental tissue to obtain the specimen with negative pressure by the syringe.

The transabdominal chorionic villus sampling technique generally utilizes a freehand technique with continuous ultrasound guidance, similar to amniocentesis or cordocentesis. Local anesthetic may be considered. A 19- or 20-gauge spinal needle is used for the transabdominal technique, while other needle options include a 2-needle set with an outer gauge of 18. The needle is moved back and

forth (5–10 movements) in the placental tissue to obtain the specimen with negative pressure by the syringe.

Rhesus prophylaxis is given if the woman is known to be Rhesus negative according to SOGC guidelines.¹¹ Patients are generally requested to have limited activity for 12 to 24 hours following the CVS procedure, but the efficacy of decreased activity in reducing the risk of pregnancy loss has not been well studied.

The transcervical technique with the biopsy forceps may be used for most placental locations, the transcervical technique with the catheter may be used for posterior placental locations, and the transabdominal technique is better suited for fundal and anterior placental locations. Both the transcervical and transabdominal technique usually obtain 5 to 25 mg of chorionic tissue. This adequate amount of chorionic villus tissue is generally obtained with 1 aspiration but 2 attempts do not increase the risk of post-procedure loss.⁴²

Both transabdominal and transcervical chorionic villus sampling have similar accuracy.⁴³ The transcervical technique is associated with a greater risk of post-procedural spotting or minimal bleeding (10%–20%)⁴⁴ while the transabdominal technique has increased uterine discomfort and cramps.⁴⁵ Infection has not been identified as a significant factor in the large number of patients having transcervical procedures.⁴²

Some genetic centres will use CVS techniques for both singleton and twin pregnancies. The safety and accuracy of CVS and twins is reported by a small number of centres.^{46,47} Separate instruments should be used when sampling multiple pregnancies.

ADVANTAGES OF CVS

The major advantage of CVS is the earlier gestational age at sampling, affording earlier results. If a chromosomal or DNA abnormality is detected and pregnancy termination is requested, some of the physical and emotional stresses of pregnancy termination may be less than when termination follows amniocentesis at a later gestational age. Secondly, specific molecular diagnoses with DNA may be extracted directly from the villi, allowing an earlier result without cell culturing for these genetic disorders. Thirdly, direct chromosomal analysis may be used in certain situations for rapid results in less than 24 hours by either cytogenetic or fluorescent insitu hybridization (FISH) techniques.

DISADVANTAGES AND RISKS OF CVS

A) Confined Placental Mosaicism

Confined placental mosaicism, a discrepancy between the chromosomes in the chorionic and fetal tissues, is a biologic placental factor which is present in 1% to 2% of

pregnancies.^{48–50} Although this finding is usually limited to the placental tissue and is not usually present in the fetus, additional amniocentesis should be offered for further evaluation. The additional procedure may increase pregnancy complication risks. Clinical effects of the confined placental mosaicism can vary depending on the specific chromosome involved. The concerns that need to be considered in this situation are uniparental disomy and risks of intrauterine growth restriction and fetal death associated with placental dysfunction.

B) Maternal Contamination

Contamination by maternal decidual tissue is possible, but this potential problem can be minimized with very careful attention to cleaning or stripping of the chorionic villi of maternal decidual cells under the dissecting microscope prior to tissue culturing. This has not been a significant problem in most cytogenetic laboratories with long-term experience in CVS.^{51,52}

C) Pregnancy Loss

The background risk of spontaneous pregnancy loss in the advanced maternal age group, after ultrasound has confirmed a viable pregnancy at 10 weeks gestational age when no procedure is undertaken, is estimated at 2% to 3%.⁵³ The CVS procedure adds approximately 1% to 2% above the background in comparison to the 0.5 to 1% risk for amniocentesis.^{17,54,55} Vaginal bleeding occurring prior to the procedure increases the risk of pregnancy loss following CVS by either transcervical or transabdominal route. The risk of pregnancy loss increases with the number of attempts needed to obtain the chorionic tissue and should be limited to 2 attempts. Uterine and placental location may alter procedural risk factors depending on the CVS technique used. Uterine fibroids may cause some additional risks of technique success and pregnancy loss. While the risks associated with transcervical technique were once thought to be double those of transabdominal technique,^{26,27,56,57} more recent evidence demonstrates similar rates of spontaneous post-procedure pregnancy loss.^{58–61}

D) Limb or Facial Anomalies

The risk of limb or facial anomalies is higher if CVS is done at a gestational age earlier than nine weeks. CVS is generally restricted to greater than or equal to 10 weeks gestational age in most centres. The incidence of transverse limb defects (minor or major) in the general population is estimated at nine in 10 000 live births. One-third of these anomalies may be due to a vascular disruption sequence event which may be associated with a CVS procedure. The risk of a limb or facial abnormality related to the CVS procedure could be as high as one in 3000 fetuses.^{62–68} A recent report from the World Health Organization (WHO)

registry concluded that CVS is not associated with an increased risks for fetal loss or anomalies.⁶⁹

REFERENCES

- Krantz DA, Larsen JW, Buchanan PD, Macri JN. First trimester Down syndrome screening: free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Am J Obstet Gynecol* 1996;174:612–6.
- Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. *Lancet* 1998;352:343–6.
- Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome based on tests performed during the first and second trimesters. *N Engl J Med* 1999;341:461–7.
- Krantz DA, Hallahan TW, Orlandi F, Buchanan P, Larsen JW Jr., Macri JN. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstet Gynecol* 2000;96:207–13.
- DeBiasio P, Siccardi M, Volpe G, Famularo L, Santi F, Canini S. First-trimester screening for Down syndrome using nuchal translucency measurements with free beta-hCG and PAPP-A between 10 and 13 weeks of pregnancy—the combined test. *Prenat Diagn* 1999;19:360–3.
- Wapner R, Thorn E, Simpson JL, Pergament E, Silver R, Filkins K, et al. First-trimester screening for trisomies 21 and 18. *N Eng J Med* 349;15:1405–13.
- Malone FD, Wald NJ, Canick JA, Ball RH, Nyberg DA, Comstock CH, et al. First and second-trimester evaluation of risk (FASTER) trial: principal results of the NICHD multicentred Down syndrome screening study [abstract]. *Am J Obstet Gynecol* 189;6:S56.
- Marthin T, Liedgren S, Hammar M. Transplacental needle passage and other risk-factors associated with second trimester amniocentesis. *Acta Obstet Gynecol Scand* 1997;76:728–32.
- Tabor A, Bang J, Norgaard-Pedersen B. Feto-maternal haemorrhage associated with genetic amniocentesis: results of a randomized trial. *Br J Obstet Gynaecol* 1987;94:528–34.
- Hess LW, Anderson RL, Golbus MS. Significance of opaque discolored amniotic fluid at second trimester amniocentesis. *Obstet Gynecol* 1986;67(suppl):44–6.
- Fung Kee Fung K, Eason E, Crane J, Armson A, De La Ronde S, Farine D, et al. SOGC Clinical Practice Guidelines. Prevention of RH Alloimmunization. *J Obstet Gynaecol Can* 2003;25(9):765–73.
- National Institute of Child Health and Human Development, National Registry for Amniocentesis Study Group. Mid-trimester amniocentesis for prenatal diagnosis: safety and accuracy. *J Am Med Assoc* 1976;236:1471–6.
- Medical Research Council of Canada. Diagnosis of genetic disease by amniocentesis during the second trimester of pregnancy. Report No. 5. Ottawa: Medical Research Council of Canada; 1977.
- Working Party on Amniocentesis. An assessment of the hazards of amniocentesis. *Br J Obstet Gynaecol* 1978;85:1–4.
- Hunter AG, Thompson D, Speevak M. Midtrimester genetic amniocentesis in eastern Ontario: a review from 1970 to 1985. *J Med Genet* 1987;24:335–43.
- Tabor A, Madsen M, Obel EB, Philip J, Bang J, Noergard-Peterson B. Randomized controlled trial of genetic amniocentesis in 4606 low-risk women. *Lancet* 1986;1(8493):1287–93.
- Canadian Collaborative CVS-Amniocentesis Clinical Trial Group. Multicentre randomised clinical trial of chorion villus sampling and amniocentesis. First Report. *Lancet* 1989;1(8628):1–6.
- The Canadian Early and Mid-trimester Amniocentesis Trial (CEMAT) Group. Randomised trial to assess safety and fetal outcome of early and mid-trimester amniocentesis. *Lancet* 1998;351:242–7.
- Eddleman K, Berkowitz R, Kharbutli Y, Malone F, Viddaver J, Flint Porter T, et al. Pregnancy loss rates after midtrimester amniocentesis: the FASTER trial. *Am J Obstet Gynecol* 2003;189(6):S111.
- D'Alton ME. Prenatal diagnostic procedures. *Semin Perinatol* 1994;18:140–62.
- Romero R, Munoz H, Gomez R, et al. Two-thirds of spontaneous abortion/fetal deaths after genetic midtrimester amniocentesis are the result of a pre-existing subclinical inflammatory process of the amniotic cavity [abstract]. *Am J Obstet Gynecol* 1995;172:261.
- Wenstrom KD, Andrews WW, Tamura T, Du Bard MB, Johnston KE, Hemstreet GP. Elevated amniotic fluid interleukin-6 levels at genetic amniocentesis predict subsequent pregnancy loss. *Am J Obstet Gynecol* 1996;175:830–3.
- Anandakumar C, Wong YC, Annapoorna V, Arulkumaran S, Chia D, Bongso A, et al. Amniocentesis and its complications. *Aust N Z J Obstet J OGC Gynaecol* 1992;32:97–99.
- Eller KM, Kuller JA. Porencephaly secondary to fetal trauma during amniocentesis. *Obstet Gynecol* 1995;85:865–7.
- Petrikovsky BM, Kaplan GP. Fetal responses to inadvertent contact with the needle during amniocentesis. *Fetal Diagn Ther* 1995;10:83–5.
- Wilson RD, Johnson J, Windrim R, Dansereau J, Singer J, Winsor EJT, et al. The early amniocentesis study: a randomized clinical trial of early amniocentesis and midtrimester amniocentesis. II. Evaluation of procedure details and neonatal congenital anomalies. *Fetal Diagn Ther* 1997;12:97–101.
- Sundberg K, Bang J, Smidt-Jensen S, Brocks V, Lundsteen C, Parner J, et al. Randomized study of risk of fetal loss related to early amniocentesis versus chorionic villus sampling. *Lancet* 1997;350(9079):697–703.
- Anderson RL, Goldberg JD, Golbus MS. Prenatal diagnosis in multiple gestation: 20 years' experience with amniocentesis. *Prenat Diagn* 1991;11:263–70.
- Taylor MJ, Fisk NM. Prenatal diagnosis in multiple pregnancy. *Baillières Clin Obstet Gynaecol* 2000;14:663–75.
- Sebire NJ, Noble PL, Odibo A, Malligianni P, Nicolaides KH. Single uterine entry for genetic amniocentesis in twin pregnancies. *Ultrasound Obstet Gynecol* 1996;7:26–31.
- Bahado-Singh R, Schmitt R, Hobbins JC. New technique for genetic amniocentesis in twins. *Obstet Gynecol* 1992;79:304–7.
- Buscaglia M, Chisoni L, Bellotti M, Marconi AM, Zamperini P, Stripparo L, et al. Genetic amniocentesis in biamnionic twin pregnancies by a single transabdominal insertion of the needle. *Prenat Diagn* 1995;15:17–9.
- Toth-Pal E, Papp C, Beke A, Ban Z, Papp Z. Genetic amniocentesis in multiple pregnancy. *Fetal Diagn Ther* 2004;19:138–44.
- Pruggmayer M, Baumann P, Schutte H, Osmers R, Bartels I, Jovanovich V, et al. Incidence of abortion after genetic amniocentesis in twin pregnancies. *Prenat Diagn* 1991;11:637–40.
- Ghidini A, Lurch L, Hicks C, Alvarez M, Lockwood CJ. The risk of second-trimester amniocentesis in twin gestations: a case-control study. *Am J Obstet Gynecol* 1993;169:1013–6.
- Wilson RD, Kent NE, Johnson J, Bebbington M. Twin gestation: evidence based outcome analysis and literature review for chromosomal aneuploidy, congenital malformations, and pregnancy loss. *J Soc Obstet Gynecol Can* 1997;19:1189–200.
- Kohl S, Casey G. Twin gestation. *Mt Sinai J Med* 1975;42:523–39.
- Coleman BG, Grumbach K, Arger PH, Mintz MC, Arenson RL, Mennuti M, et al. Twin gestations: monitoring of complications and anomalies with US. *Radiology* 1987;165:449–53.

39. Pruggmayer M, Jahoda MG, Van de Pol JG, Baumann P, Holzgreve W, Karkut G, et al. Genetic amniocentesis in twin pregnancies: results of a multicenter study of 529 cases. *Ultrasound Obstet Gynecol* 1992;2:6–10.
40. Winsor EJ, Tomkins DJ, Kalousek D, Farrell S, Wyatt P, Fan YS, et al. Cytogenetic aspects of the Canadian early and mid-trimester amniotic fluid trial (CEMAT). *Prenat Diagn* 1999;19:620–7.
41. Philip J, Silver RK, Wilson RD, Thom EA, Zachary JM, Mohide P, et al. Late first-trimester invasive prenatal diagnosis: results of an international randomized trial. *The Am Coll Obstet Gynecol* 103;6:1164–73.
42. Wilson RD, Cho K, McGillivray B, Kalousek D, Shaw D, Baldwin V. Chorionic villus sampling: analysis of fetal losses to delivery, placental pathology and cervical microbiology. *Prenat Diagn* 1991;11:539–50.
43. Brambati B, Lanzani A, Tului L. Transabdominal and transcervical chorionic villus sampling: efficiency and risk evaluation of 2,411 cases. *Am J Med Genet* 1990;35:160–4.
44. Shime J, Benzie R, Mohide P, Wilson D, Natale R, Johnson J. Canadian multicenter randomized clinical trial of chorion villus sampling and amniocentesis: detailed obstetrical procedures and results. *Prenat Diagn* 1992;12:411–22.
45. Wilson RD. Chorionic villus sampling: a risk benefit breakdown. *Can J Diagn* 1996;13(2):43–61.
46. Wapner RJ, Johnson A, Davis G, Urban A, Morgan P, Jackson L. Prenatal diagnosis in twin gestations: a comparison between second-trimester amniocentesis and first-trimester chorionic villus sampling. *Obstet Gynecol* 1993;82:49–56.
47. De Catte L, Liebaers I, Foulon W, Bonduelle M, Van Assche E. First trimester chorionic villus sampling in twin gestations. *Am J Perinatol* 1996;13:413–7.
48. Kalousek DK, Dill FJ, Pantzar T, McGillivray BC, Yong SL, Wilson RD. Confined chorionic mosaicism in prenatal diagnosis. *Hum Genet* 1987;77:163–7.
49. Kalousek DK, Howard-Peebles PN, Olson SB, Barrett IJ, Dorfmann A, Black SH, et al. Confirmation of CVS mosaicism in term placentae and high frequency of intrauterine growth retardation association with confined placental mosaicism. *Prenat Diagn* 1991;11:743–50.
50. Kalousek DK, Langlois S, Barrett I, Yam I, Wilson DR, Howard-Peebles PN, et al. Uniparental disomy for chromosome 16 in humans. *Am J Hum Genet* 1993;52:8–16.
51. Rudd N, Cox D. Prenatal diagnosis: chorionic villus sampling (CVS) update. *Bull Hared Dies Program Alberta* 1989;8:13–6.
52. Ledbetter DH, Martin AO, Verlinsky Y, Pergament E, Jackson L, Yang-Feng T, et al. Cytogenetic results of chorionic villus sampling: high success rate and diagnostic accuracy in the United States collaborative study. *Am J Obstet Gynecol* 1990;162(2):495–501.
53. Wilson RD, Kendrick V, Wittmann BK, McGillivray B. Spontaneous abortion and pregnancy outcome following normal first trimester ultrasound. *Obstet Gynecol* 1986;67:352–5.
54. Rhoads GG, Jackson LG, Schlesselman SE, de la Cruz FF, Desnick RJ, Golbus MS, et al. The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. *N Eng J Med* 1989;320:609–63.
55. Medical Research Council European trial of chorion villus sampling. MRC working party on the evaluation of chorion villus sampling. *Lancet* 1994;337:1491–9.
56. Silver RK, MacGregor SN, Muhlback LH, Kambich MP, Ragin A. A comparison of pregnancy loss between transcervical and transabdominal chorionic villus sampling. *Obstet Gynecol* 1994;83:657–60.
57. Chueh JT, Goldberg JD, Bohlferd MM, Golbus MS. Comparison of transcervical and transabdominal chorionic villus sampling loss rates in nine thousand cases from a single center. *Am J Obstet Gynecol* 1995;173:1277–82.
58. Papp C, Beke A, Mezei G, Toth-Pal E, Papp Z. Chorionic villus sampling: a 15-year experience. *Fetal Diagn Ther* 2002;17:218–27.
59. Alfirevic Z, von Dadelszen P. Instruments for chorionic villus sampling for prenatal diagnosis (Cochrane Review). In the Cochrane Library, Issue 3, 2004. Chichester, UK: John Wiley & Sons, Ltd.
60. Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis (Cochrane Review). In the Cochrane Library, Issue 3, 2004. Chichester, UK: John Wiley & Sons, Ltd.
61. Borrell A, Fortuny A, Lazaro L, Costa D, Seres A, Pappa S, et al. First-trimester transcervical chorionic villus sampling by biopsy forceps versus midtrimester amniocentesis: a randomised controlled trial project. *Prenat Diagn* 1999;19:1138–42.
62. Froster-Iskenius UG, Baird PA. Limb reduction defects in over one million consecutive live births. *Teratology* 1989;39:127–35.
63. Firth HV, Boyd PA, Chamberlain P, MacKenzie IZ, Lindenbaum RH, Huson SM. Severe limb abnormalities after chorion villus sampling at 56–66 days' gestation. *Lancet* 1991;337:762–3.
64. Burton BK, Schultz CJ, Burd LI. Limb abnormalities associated with chorionic villus sampling. *Obstet Gynecol* 1992;79:726–30.
65. Brambati B, Simoni G, Travi M, Danesino C, Tului L, Privitera O, et al. Genetic diagnosis by chorionic villus sampling before 8 gestational weeks: efficiency, reliability, and risks on 317 completed pregnancies. *Prenat Diagn* 1992 oct;12(10): 789–99.
66. Holmes LB. Limb deficiency defects among 125,000 newborn infants. *Am J Hum Genet* 1992;51(sup):A18
67. Stoll C, Alembik Y, Dott B, Roth MP. Risk factors in limb reduction defects. *Paediatr Perinat Epidemiol* 1992;6:323–38.
68. Froster UG, Jackson L. Limb defects and chorionic villus sampling: results from an international registry, 1992–94. *Lancet* 1996;347:489–94 .
69. WHO/PAHO Consultation on CVS. Evaluation of chorionic villus sampling safety. *Prenat Diagn* 1999;19:97–99.
70. Woolf SH, Battista RN, Angerson GM, Logan AG, Eel W. Canadian Task Force on the Periodic Health Exam. Ottawa: Canada Communication Group; 1994. p. xxxvii.