Prenatal Diagnosis Procedures and Techniques to Obtain a Diagnostic Fetal Specimen or Tissue: Maternal and Fetal Risks and Benefits

Abstract

Objective: To provide maternity care providers and their patients with current evidence-based guidelines for maternal risk/benefit counselling for a prenatally identified at-risk pregnancy that requires ultrasound-guided prenatal diagnostic procedures and/or techniques for a genetic diagnosis and for subsequent pregnancy management decisions on questions such as level of obstetrical care provider, antenatal surveillance, location of care and delivery, and continuation or termination of pregnancy. This guideline is limited to maternal risk/benefit counselling and pregnancy management decisions for women who require, or are considering, an invasive ultrasound-guided procedure or technique for prenatal diagnosis.

Patient population: Pregnant women identified as having an increased risk of a fetal genetic abnormality secondary to the process of established prenatal screening protocols (maternal serum ± imaging, high-risk cell-free DNA results, abnormal diagnostic fetal imaging, or a positive family history of an inherited condition). These women may require or request counselling about pregnancy risks and benefits of an invasive ultrasound-guided procedure to determine the etiology, diagnosis, and/or pathology for the possible fetal anomaly or anomalies.

Evidence: Published literature was retrieved through searches of Medline, PubMed, and the Cochrane Library in and prior to June 2014 using an appropriate controlled vocabulary (prenatal diagnosis, amniocentesis, chorionic villi sampling, cordocentesis) and key words (prenatal screening, prenatal genetic counselling, post-procedural pregnancy loss rate). Results were restricted to systematic reviews, randomized control trials/controlled clinical trials, and observational studies written in English and published from January 1985 to June 2014. Searches were updated on a regular basis and incorporated in the guideline to June 2014. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology-related agencies, clinical practice guideline collections, clinical trial registries, and national and international medical specialty societies.

Key words: Prenatal diagnosis, prenatal genetic counselling, prenatal procedure risk, prenatal procedure benefit, amniocentesis, chorionic villi sampling, cordocentesis
INTRODUCTION

The traditional gold standard prenatal diagnostic results for the fetus are obtained through genetic analysis of pregnancy-related tissues from CVS, AC, or cordocentesis. Currently, maternal serum cfDNA is used for genetic screening, which is followed by traditional prenatal diagnostic techniques when a screen is positive. However, in the future, maternal serum cfDNA may itself come to be used for fetal diagnosis. The risks and benefits for the mother and fetus differ with invasive (traditional) and non-invasive (new) approaches.1–9

While the scope of prenatal genetic diagnosis is usually based on the identification of fetal karyotype abnormalities, other analyses of specific genetic mutations are also possible using amniocytes, chorionic villus, or fetal blood. Maternal serum cfDNA molecular technology has potential diagnostic capability, but at the present time is generally restricted to fetal sexing, fetal Rh typing, and screening for trisomies 21, 18, and 13. Other fetal genetic mutations have been identified from maternal serum cfDNA, but only on the basis of a case-by-case genetic differential diagnosis or when a specific family mutation has been identified.

Prenatal diagnostic counselling begins with collecting the patient’s family history, ethnic background, past genetic, obstetrical, medical, and surgical history, and the indication for diagnostic fetal testing, and learning about the personal values and needs of the woman and her family. Parental karyotyping may be required for family or personal history of recurrent pregnancy loss or when there is a recognized family history for translocation carrier risks. Molecular genetic testing or referral for genetic assessment may be required when one of the parents presents characteristics suspicious of an undiagnosed genetic syndrome. Maternal and paternal

Table 1. Key to evidence statements and grading of recommendations, using the ranking of the Canadian Task Force on Preventive Health Care

<table>
<thead>
<tr>
<th>Quality of evidence assessment*</th>
<th>Classification of recommendations†</th>
</tr>
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<tbody>
<tr>
<td>I: Evidence obtained from at least one properly randomized controlled trial</td>
<td>A. There is good evidence to recommend the clinical preventive action</td>
</tr>
<tr>
<td>II-1: Evidence from well-designed controlled trials without randomization</td>
<td>B. There is fair evidence to recommend the clinical preventive action</td>
</tr>
<tr>
<td>II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group</td>
<td>C. The existing evidence is conflicting and does not allow to make a recommendation for or against use of the clinical preventive action; however, other factors may influence decision-making</td>
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<tr>
<td>II-3: Evidence obtained from comparisons between times or places with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of treatment with penicillin in the 1940s) could also be included in this category</td>
<td>D. There is fair evidence to recommend against the clinical preventive action</td>
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<tr>
<td>III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees</td>
<td>E. There is good evidence to recommend against the clinical preventive action</td>
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<td></td>
<td>F. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making</td>
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</table>

*The quality of evidence reported in these guidelines has been adapted from The Evaluation of Evidence criteria described in the Canadian Task Force on Preventive Health Care.60
†Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the Canadian Task Force on Preventive Health Care.60

Values: The quality of evidence in this document was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care (Table 1).

Health benefits, side effects, and risks: Patient informed consent, knowledge translation, genetic prenatal risk assessment, anxiety relief, anxiety creation, advocacy, understanding or limitation for fetal testing, pregnancy management choice, pregnancy complication or loss, timely and improved care for birth of a neonate with recognized morbidity.

Recommendations

1. The health care provider should counsel the at-risk pregnant woman on the different levels of genetic fetal testing in order for her to have a clear understanding and expectation of the level of testing and type of results that are offered. (III-B)
2. As part of the informed consent process, the health care provider should review with the at-risk pregnant woman the risks and benefits of in utero genetic diagnostic techniques associated with fetal genetic testing options. (III-A)
3. During risk/benefit counselling, the health care provider should advise that the best estimate of the pregnancy loss rate related to:
   a. amniocentesis is 0.5% to 1.0% (range 0.17 to 1.53%) (I)
   b. chorionic villus sampling is 0.5% to 1.0% (I) and
   c. cordocentesis or percutaneous umbilical blood sampling is 1.3% for fetuses with no anomalies and 1.3% to 25% for fetuses with single or multiple anomalies or intrauterine growth restriction. (II-2A)
factors (genetics, family, ethnic, reproductive ages, and personal health history) that may add to the pregnancy risk are summarized in Table 2.9

Pre-procedural counselling requires a very clear understanding by both the patient and the provider of the level of genetic testing or diagnosis that is offered or requested. The patient needs a clear explanation, at a level appropriate to her education, literacy, and language skills, of the screening test or fetal anomaly results that have led her to consider prenatal diagnostic fetal testing, so that she can provide informed consent.9–24 The level and depth of the counselling care and information provided also depend on the expertise of the provider.1–9

Once the criteria for offering prenatal invasive testing for an at-risk pregnancy have been met, counselling should include a verbal description, illustrated with diagrams or images, of the most appropriate prenatal procedure for the recommended or required diagnostic genetic testing.

The evidence-based rates for spontaneous (no procedure) pregnancy loss summarized in Table 3 may be used during procedure-related pregnancy loss counselling.25–34

Test results and follow-up planning and counselling require a clear description of the time factors related to the diagnostic testing and its results.22

This guideline is limited to the genetic diagnostic procedures of CVS, AC, and cordocentesis/PUBS and intended to assist providers in counselling women about targeted fetal genetic testing after a positive obstetrical screening test or the ultrasound identification of fetal anomalies. Routine pregnancy counselling and the offer of prenatal genetic screening have been previously reviewed and published in the SOGC Guideline, “Counselling Considerations for Prenatal Genetic Screening,”22 and two separate guidelines for obstetrical aneuploidy screening in singleton and twin pregnancies.23,24

Invasive in utero prenatal diagnosis techniques include CVS, AC, PUBS, and fetal tissue sampling (skin, muscle, kidney, liver, ascites, pleural effusion, urine). Some genetic or pathologic diagnostic results may be obtained by more than one technique; for example, fetal karyotype results can be obtained from CVS, AC, and PUBS, but each technique may be provided at different gestational ages.10–21

**What level of genetic testing analysis does the patient need or want? A risk assessment summary**

Maternal and paternal testing need to be specifically directed but are based on past family, ethnic, and obstetrical outcomes history and present pregnancy indications (Table 2).9

The available prenatal genetic fetal testing levels must be clear to the patient because they include details ranging from standard or basic to increased levels of molecular complexity. The following levels of testing should be explained:

a. numerical assessment of chromosomes 13, 18, 21, X, and Y by quantitative fluorescence-polymerase chain reaction or FISH;

b. fetal karyotype testing for only the number of chromosomes or chromosome pairs and detection of large chromosome rearrangements, deletions, or duplications;

c. fetal karyotype testing (as in the previous point) with specific directed testing for molecular chromosomal deletions or duplications related to past obstetrical or family history or present fetal anomaly:

Deletions (interstitial p or q chromosome arm location; terminal and subtelomeric location) should be discussed with examples of their associated anomalies, such as:

- del(22q11.2) Di George syndrome: cardiac anomaly, thymic hypoplasia, parathyroid dysfunction, cleft palate, distinctive face;
- del(7q11.23) Williams syndrome: cardiac anomaly, characteristic facies, developmental delay; and
- del(17p13.3) Miller-Dieker syndrome: cardiac anomaly, omphalocele, joint contractures, characteristic facies.

Duplications (interstitial, direct “abab” or inverted “abba”, and terminal and subtelomeric location) should be discussed, including fetal karyotype (as above) with use of an expanded detailed chromosomal microarray (array genomic hybridization) when fetal anomalies are identified.35–37

- d. prenatal chromosomal microarray identified clinically relevant deletions or duplications in 1.7% of cases with normal karyotype in a prenatal population with a positive genetic screen (maternal age or positive screen in the 1st or 2nd trimester) as the indication for conducting a prenatal karyotype:

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AC</td>
<td>amniocentesis</td>
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<tr>
<td>AF</td>
<td>amniotic fluid</td>
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<td>cfDNA</td>
<td>cell-free DNA</td>
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<td>CVS</td>
<td>chorionic villus sampling</td>
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<tr>
<td>CSCNV</td>
<td>clinically significant copy number variant</td>
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<tr>
<td>FISH</td>
<td>fluorescence in situ hybridization</td>
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<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>PUBS</td>
<td>percutaneous umbilical blood sampling</td>
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<tr>
<td>TA</td>
<td>transabdominal</td>
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<tr>
<td>TC</td>
<td>transcervical</td>
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Table 2. Taking a pre-conception history for assessment and counselling

<table>
<thead>
<tr>
<th>GENETIC HISTORY</th>
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<tbody>
<tr>
<td>A thorough pre-conception history identifies couples who are genetically at risk. When women and their partners are informed of the risks of having a baby with birth defects or a genetic disorder prior to pregnancy, they are then able to determine their options regarding a pregnancy (including contraception, gamete donation, adoption, prenatal invasive testing, or chance).</td>
</tr>
</tbody>
</table>

Family history

Construct a three-generation pedigree.
- Include assessment of genetic diseases, including muscular dystrophy, hemophilia, cystic fibrosis, fragile X syndrome, syndromic congenital heart disease, phenylketonuria, skeletal dysplasia, sickle cell anemia, hemoglobinopathies, and Tay-Sachs disease.
- Include assessment of multifactorial congenital malformations, such as spina bifida, anencephaly, cleft palate and cleft lip, hypospadias, and congenital heart disease.
- Include assessment of familial diseases with a major genetic component, such as developmental disability, premature atherosclerosis, diabetes mellitus, psychosis, epileptic disorders, hypertension, rheumatoid arthritis, deafness, and severe refractive disorders of the eye.

Ethnic history

Establish risks associated with age (e.g., women under age 15 or over age 35 may carry increased biological risks).

Age

Establish risks associated with age (e.g., women under age 15 or over age 35 may carry increased biological risks).

HEALTH HISTORY

Chronic conditions

Assess the presence of chronic conditions that can affect a woman’s ability to conceive, as well as the use of medications in treatment of chronic disease and their potential effect on pregnancy such as teratogenicity.
- To be considered: diabetes mellitus, anemia, thyroid disorders, gynaecological disorders, hyperphenylalaninemia, asthma, sexually transmitted infections, heart disease, hypertension, deep venous thrombosis, kidney disease, systemic lupus erythematosus, epilepsy, hemoglobinopathies, cancer, seizure disorders, tuberculosis, rheumatoid arthritis, and mental health/psychiatric disorders.

Infectious conditions

Identify women who are rubella- or varicella-susceptible. If they are not actively attempting pregnancy, offer a vaccination.

Identify and counsel women at risk for hepatitis B. Routine pre-conception testing of all women with hepatitis B is not currently recommended.

Counsel women to avoid exposure to cat feces and raw and undercooked meats. Routine serologic testing for toxoplasmosis in the pre-conception period or in pregnancy is not recommended.

Evaluate the woman and her partner for exposure to sexually transmitted infection (e.g., chlamydia, HIV, gonorrhea, syphilis).

Reproductive history

Collect information about menstrual, contraceptive, and sexual histories; infertility; abnormal Pap smears; and in utero exposure to diethylstilbestrol.

Discuss past obstetric history, including early miscarriages; number of pregnancies; type of birth; length of labour; and specific complications, such as premature labour or delivery, gestational diabetes, pregnancy-induced hypertension, and postpartum depression.

Discuss menstrual difficulties, specifically excessive cyclic bleeding, amenorrhea, and oligomenorrhea.

Discuss gynaecological disease, such as endometriosis and pelvic inflammatory disease.

Lifestyle assessment

Assess lifestyle issues, including nutrition, physical activity, prescription and over-the-counter drug use, other substance use, and environmental exposures, current and past.

This enhanced genetic analysis requires continued directed research during its introduction as part of the routine evaluation. In the same study, the prenatal chromosomal microarray identified an additional 6.0% of cases with a clinically relevant deletion or duplication that was not identified by the standard karyotype when fetal anomalies were the indication for a prenatal karyotype. Ultrasound-detected fetal anomalies from the NICHD Microarray Trial were analyzed according to the additional microarray genetic pathology and the fetal organ system involved. For the 1082 fetuses with anomalies, 752 had a normal karyotype. Clinically significant copy number variants were present in 61 of the euploid fetuses (8.1%). CSCNVs were present in 13% of fetuses with multiple system anomalies compared with 3.6% of fetuses with no anomalies ($P < 0.001$). For isolated anomalies, the CSCNVs were nominally significant for renal ($P = 0.04$) and cardiac ($P = 0.01$). Other anomalies were small in number and did not meet statistical significance.

e. fetal karyotype with more directed complex or detailed genetic testing because of past reproductive outcome, family history, extended and complex fetal differential diagnosis based on prenatal findings, or personal informed choice:

Evaluation data on the use of whole-exome sequencing in pediatric patients with a suspected Mendelian disorder is lending support for the use of this new technology in the prenatal population. In a cohort of 250 children (80% with a neurological phenotype), 86 mutated alleles were found that were highly likely to be causative in 62 of the 250 patients. The result indicated a 25% molecular diagnostic rate (95% CI 20 to 31) with 33 autosomal dominant, 16 autosomal recessive, and 9 X-linked conditions.

A prenatal invasive diagnostic procedure counselling checklist (Table 4) has been created to assist the maternity care provider with the primary stages of counselling prior to referral, regional or tertiary centre counselling, and informed consent.

### What is the possible etiology for the screen positive result or the structural fetal pathology leading to the consideration of an invasive diagnostic procedure?

Correct gestational dating is required for accurate genetic assessment and evaluation. Butt et al. provided evidence-based recommendations related to the timing (1st and 2nd trimester) of dating ultrasounds.

Ultrasound, ideally performed at 18 to 22 weeks’ gestation, is the primary imaging screening and diagnostic tool recommended for fetal anatomy, number, and growth. MRI is used as a second-tier imaging modality, following an abnormal ultrasound; it is usually performed after 22 weeks’ gestation.

Major fetal congenital anomalies (malformation, disruption, deformation, dysplasia) occur in an estimated 5% of all live births (3% are identifiable prenatally and 2% at birth or
### Table 4. Checklist: reproductive genetics for in utero diagnostic prenatal testing

<table>
<thead>
<tr>
<th>Name:</th>
<th>Date of birth:</th>
<th>Maternal age at expected date of delivery:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paternal age at expected date of delivery:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestations:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Term pregnancies:</td>
<td></td>
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<tr>
<td>Preterm pregnancies:</td>
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<td>Spontaneous abortions:</td>
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<td>Therapeutic abortions:</td>
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<tr>
<td>Live births:</td>
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<tr>
<td>Stillbirths:</td>
<td></td>
<td></td>
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<tr>
<td>Neonatal deaths:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Important maternal co-morbidities:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paternal co-morbidities:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assisted reproductive technology:</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

1. Indication for invasive prenatal testing
   - Past obstetrical history (fetal chromosomal anomaly/genetic syndrome)
     Specify:
   - Positive family history (translocation carrier; genetic carrier AR/AD/XL inheritance)
     Specify:
   - Positive aneuploidy screening test (first/second trimester positive for nuchal translucency; maternal age > 35)
     Specify:
   - Fetal anomalies identified by ultrasound imaging
     Specify:

2. Depth/complexity of fetal testing discussed in patient informed consent counselling
   Genetic complexity, levels I–V
   - I. Fetal karyotype only (numerical 13, 18, 21, X, Y by QF-PCR or FISH; standard karyotype only)
   - II. Fetal karyotype plus selected molecular deletion/duplication testing
     Specify molecular deletion/duplication test:
   - III. Fetal karyotype plus array comparative genomic hybridization
   - IV. Fetal karyotype plus whole genome sequencing
   - V. Fetal sexing only (molecular/ultrasound)
   - Other: amniotic fluid testing

3. Procedural risk counselling (procedure and gestational age timing described)
   - Amniocentesis; pregnancy loss risk 0.5% to 1.0% (range 0.17 to 1.53%)
   - Chorionic villus sampling: pregnancy loss risk 0.5% to 1.0%
   - Cordocentesis: pregnancy loss rate with no anomalies 1.3% with fetal anomalies 1.3% to 25%

4. Pregnancy management options
   - Consultation required: obstetrics, maternal-fetal medicine, neonatology, medical genetics
   - Consultation and transfer of care for delivery required: obstetrics, maternal–fetal medicine
   - Pregnancy termination (if under consideration)
   - Continuation of pregnancy (regardless of diagnostic findings)
   - City and hospital for delivery planning:

5. Follow-up post-delivery planning
   - Autopsy discussion
   - In-depth reproductive genetic counselling
   - Pre-conception planning visit recommended

What is the possible etiology for the screen positive result or the structural fetal pathology leading to the consideration of an invasive diagnostic procedure?

AR: autosomal recessive; AD: autosomal dominant; XL: X-linked; QF-PCR: quantitative fluorescence-polymerase chain reaction
during the first year of life, as some anomalies will have a functional component with no obvious structural change. Minor structural anomalies are becoming more identifiable with improved ultrasound technology, allowing for more detailed facial, CNS, and cardiac imaging.

The most commonly recognized etiologies for fetal anomalies are chromosomal abnormalities, teratogenic exposure (drugs, chemical, infectious), maternal co-morbidities (maternal age > 35 years, diabetes, epilepsy, hypertension), deformations or disruptions (structural uterine anomalies, oligohydramnios, monochorionic twinning abnormalities) and placental abnormalities. Confined placental mosaicism is normally present in 1% to 2% of placentas, where it is limited to the placenta and the fetus is chromosomally numerically normal but may have a genetic anomaly such as uniparental disomy. This placental and embryonic biological discordance will have a possible impact on invasive CVS trophoblastic analysis. True fetal mosaicism is rare, so AC will sometimes, be affected, but minimally.

The pregnant woman identified to have an aneuploidy screen positive result or an ultrasound with a fetal anomaly or anomalies requires reproductive genetic counselling so that she has a clear understanding of her a priori risk assessment for fetal pathology and outcome, which will allow her to make an informed choice in regard to in utero diagnostic testing (Tables 2 to 4).

**Recommendation**

1. The health care provider should counsel the at-risk pregnant woman on the different levels of genetic fetal testing in order for her to have a clear understanding and expectation of the level of testing and type of results that are offered. (III-B)

**Techniques 101: for patient and family risk counselling and discussion of technique**

All of the in utero diagnostic techniques (AC, CVS, PUBS) are done under continuous ultrasound guidance, thereby minimizing any unintended fetal damage or injury. Prophylactic antibiotics are not required for the procedure. Patients are recommended to consider decreased physical activity for 12 to 24 hours after the procedure, but bed rest is not required.

The in utero prenatal diagnosis techniques of AC and CVS are used for both singleton and twin pregnancies, and PUBS is used in singleton and dichorionic twin pregnancies.

AC is the most common in utero prenatal testing technique, and it is recommended for use after 15 weeks’ gestation, usually with a 22-gauge spinal needle with stylet to obtain the specimen of AF. During AC, placental puncture with the needle should be avoided if possible. Sterile technique is recommended, with the use of abdominal antiseptic cleaning, gloves, sterile drapes, and a sterile ultrasound probe cover. Maternal local anaesthetic is not usually required. A single needle is usually inserted, and the AF volume removed is 15 to 25 cc depending on the fetal testing required. Testing is usually from amniocytes (fetal origin from skin or bladder) for chromosome analysis and from protein, biochemical, or enzymatic analysis of the AF supernatant. Results are usually available after 1 to 3 weeks. Spotting, bleeding, or fluid leakage after AC is estimated at 1% to 5% and is usually limited with decreased activity.

Early AC at 12 to 15 weeks’ gestation is not recommended due to an increased risk of pregnancy loss and fetal talipes (club foot) secondary to temporary or intermittent oligohydramnios.

CVS is the recommended first trimester in utero technique. TCCVS (at 10 to 13+6 weeks’ gestation) is an ultrasound-guided technique using a flexible catheter and syringe suction or metal biopsy forceps to obtain placental tissue. TACVS (at 10 to 36 weeks’ gestation) is an ultrasound-guided technique using an 18- to 20-gauge needle and syringe suction to obtain the placental tissue. Because of the larger needle gauge used in TACVS and the aspirating needle movement within the placenta, local anaesthesia may be required depending on patient need. Karyotype results and time to result availability are similar using either approach. TCCVS has an estimated post-procedural risk of vaginal spotting or minimal bleeding of 10% to 20%, while TACVS has more post-procedural uterine discomfort and cramping.

Four Cochrane systematic reviews have evaluated various aspects of the invasive prenatal diagnosis techniques:

Alfirevic et al. concluded that “second trimester amniocentesis is safer than early amniocentesis or transcervical CVS, and is the procedure of choice for second trimester testing. Transabdominal CVS should be regarded as the procedure of first choice when testing before 15 weeks gestation. Diagnostic accuracy of different methods could not be assessed adequately because of incomplete karyotype data in most studies.”

Mujezinovic and Alfirevic concluded that “in general, women that 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 anaesthetics, leg rubbing or subfreezing the needle for pain reduction during procedure.”
Mujezinovic and Alfirevic examined technique variations or modifications for reducing the risks from AC or CVS, and found that, “in the absence of clear evidence, the operators should continue to use methods and technique modifications with which they are most familiar”.

Young et al concluded that “for transcervical CVS, the evidence is not strong enough to support a change in practice for clinicians who have become familiar with a particular technique. Based on current evidence, there is no difference in clinically important outcomes with the use of a continuous compared with a discontinuous negative pressure needle aspiration system.”

Cordocentesis or PUBS is usually performed after 18 weeks’ gestation and is used for both fetal diagnosis and fetal therapy (intrauterine fetal transfusion). It is a continuous ultrasound-guided technique with a 20- to 22-gauge needle being directed, preferentially, into the umbilical cord vein. Puncture of the umbilical artery can cause umbilical arterial constriction with possible fetal cardiac dysfunction. Needle puncture sites are variable and depend upon the provider's preference at the fixed placental umbilical cord insertion site, the fetal intrahepatic vein, or a free loop of umbilical cord usually pinned against the fetus, placenta, or uterine wall to allow venipuncture. A recent systematic review of the technique details the risks and benefits of this technique usually offered by trained and experienced providers.

**Invasive prenatal diagnosis technique: risk/benefit summaries**

Table 5 summarizes risk/benefit studies of AC. Additional risk details for AC include:

- procedure-related loss difference with maternal age > 35 years:14:
  - < 24 weeks 0.17% (0.37; 0.20)
  - < 28 weeks 0.50% (1.37; 0.87)
- singleton loss rates:19
  - total post amniocentesis pregnancy loss: 1.9% (1.4 to 2.5)
  - pregnancy loss < 24 weeks post/ameniocentesis: 1.3% (1.0 to 1.7)
- total post-procedural rates of:
  - miscarriage: 1.2% to 1.5%
  - intrauterine death: 0.5% to 0.9%
  - termination: 2.5% to 5.7%
  - live birth: 92.1% to 95.5%

- maternal age at procedure and total post-procedural loss rates:17:
  - age < 30: 1.5%
  - age 30 to 34: 1.3%
  - age > 34: 1.4%
- twin loss rates:9:
  - total post AC pregnancy loss: 3.07% (1.83 to 4.61)
  - pregnancy loss < 24 weeks post AC: 2.54% (1.43 to 3.96)

Table 6 summarizes risk/benefit studies of CVS. Additional details of CVS risk include:

- singleton loss rates:49
  - total post CVS pregnancy loss: 2.0% (1.4 to 2.6)
  - pregnancy loss rate < 20 weeks post CVS: 0.8% (0.2 to 1.7)
  - pregnancy loss rate < 24 weeks post CVS: 1.3% (amnio 0.9%)
- total post procedure rates of:
  - miscarriage: 1.6% to 2.4%
  - intrauterine death: 0.4% to 0.5%
  - termination: 3.8% to 10.1%
  - live birth: 87.6% to 94.3%
- maternal age at procedure and total post procedure loss rates:
  - age < 30: 1.5%
  - age 30 to 34: 1.7%
  - age > 34: 2.0%
- twin loss rates:
  - total post CVS pregnancy loss: 3.84% (2.48 to 5.47)
  - pregnancy loss < 20 weeks: 2.75% (1.28 to 4.75)

The relative risk for CVS technique in twins (TA > TC) is 2.08 (0.73 to 5.91; total fetal loss: TA 7.09% [10/141] and TC 3.94% [5/127]).

CVS operator experience and safety improved with higher annual numbers and combined TA/TC experience versus TC alone.53

Significantly increased TCCVS post-procedural pregnancy loss rates and complications are associated with the number of cervical passages: > 1 pass, OR for loss is 3.96 (P = 0.01) and for complication is 2.76 (P = 0.02).54

- twin loss rates:
  - total post CVS pregnancy loss: 3.84% (2.48 to 5.47)
  - pregnancy loss < 20 weeks: 2.75% (1.28 to 4.75)
Table 5. Amniocentesis procedure

<table>
<thead>
<tr>
<th>Indications: increased risk of fetal chromosomal or genetic pathology based on previous obstetrical or family history, maternal age, positive aneuploidy screening test, single or multiple major congenital anomalies, parental chromosomal translocation carrier</th>
<th>Singleton</th>
<th>Twin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational age range: second and third trimesters</strong></td>
<td>≥ 15 to 38 weeks’ gestation</td>
<td>≥ 15 to 38 weeks’ gestation</td>
</tr>
<tr>
<td>(Early amniocentesis at 12 to 15 weeks is not acceptable care.)</td>
<td>Overall distribution of gestational age at the time of amniocentesis from a 32 852 cohort (1996–2006)\textsuperscript{17}</td>
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<tr>
<td></td>
<td>• &lt; 15 weeks (21.6%)</td>
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</tr>
<tr>
<td></td>
<td>• ≥ 15 weeks (78.4%)</td>
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<tr>
<td><strong>Risk of miscarriage</strong> above the estimated background rate or as the loss rate (total or at a specific GA beyond procedural related affect, related to maternal age, GA at procedure, indication for procedure, provider experience)</td>
<td>Estimated total singleton procedure loss risk is 0.5% to 1.0% (range 0.17 to 1.5%)</td>
<td>Estimated “attributable” twin procedure risk\textsuperscript{46–48}:</td>
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<tr>
<td></td>
<td>Single RCT\textsuperscript{45}: Total pregnancy loss difference post-procedure was 1.0% (95% CI 0.3 to 1.5%)</td>
<td>• twin amniocentesis 2.7%</td>
</tr>
<tr>
<td></td>
<td>Post-amniocentesis loss rate (1.7%) versus spontaneous loss rate with no amniocentesis (0.7%)</td>
<td>• twin no amniocentesis 0.6%</td>
</tr>
<tr>
<td></td>
<td>Cohort summary\textsuperscript{46}: Pregnancy loss attributable to amniocentesis procedure: 0.6 to 1.0% (range 0.19 to 1.53%)</td>
<td>Systematic review\textsuperscript{20}</td>
</tr>
<tr>
<td></td>
<td>Pregnancy loss (23/632)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• OR 3.07% (95% CI 1.83 to 4.61)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fetal loss (87/1741)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• OR 4.14% (95% CI 1.91 to 7.15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meta-analysis of 2026 twin pregnancies with amniocentesis\textsuperscript{49}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• OR 2.42% (95% CI 1.24 to 4.74)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Procedure loss with chorionicity separation is very limited with no defined estimation</td>
<td></td>
</tr>
<tr>
<td><strong>Fetal anomaly disruptive risk</strong></td>
<td>No risk</td>
<td>No risk</td>
</tr>
<tr>
<td><strong>Probability of successful procedure (counselling point)</strong></td>
<td>With a skilled provider &gt; 99%, unless chorion-amnion separation occurs</td>
<td>&gt; 99%</td>
</tr>
<tr>
<td></td>
<td>But possible difference for MC and DC twins</td>
<td></td>
</tr>
<tr>
<td><strong>Time to laboratory diagnosis</strong></td>
<td>Standard time for rapid &lt; 24 hrs and culture in 1 to 3 weeks</td>
<td>Standard time for rapid &lt; 24 hrs and culture in 1 to 3 weeks</td>
</tr>
<tr>
<td><strong>Accuracy (chromosomes/aneuploidy/translocation)</strong></td>
<td>Highly accurate for large chromosomal pathology</td>
<td>Highly accurate for large chromosomal pathology</td>
</tr>
<tr>
<td><strong>Other lab-based testing</strong></td>
<td>Microarray</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Whole genome sequencing</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Lab-based findings</strong></td>
<td>Mosaicism</td>
<td>True fetal mosaicism is rare</td>
</tr>
<tr>
<td></td>
<td>AFP</td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td>AChE</td>
<td>Possible</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>Other AF products from fetal urine and respiratory sources can be measured</td>
</tr>
<tr>
<td><strong>Other post procedural risks</strong></td>
<td>AF leakage: talipes at 15 to 16 weeks: 1.7%–2.4% to 0.2%–0.8% (early amniocentesis at 12 to 15 weeks is no longer acceptable care.)</td>
<td>Background “no procedure” loss rate for twins is estimated to be higher than for singletons; probable background chorionicity loss rate is higher in MC than DC.</td>
</tr>
</tbody>
</table>

AFP: alpha-fetoprotein; AChE: acetylcholinesterase; MC monochorionic; DC: dichorionic; AF: amniotic fluid
**Table 6. CVS (TA/TC) procedures**

**Indications:** increased risk of fetal chromosomal or genetic pathology based on previous obstetrical or family history, maternal age, positive aneuploidy screening test, single or multiple major congenital anomalies, parental chromosomal translocation carrier

<table>
<thead>
<tr>
<th>Gestational age range: first to third trimester</th>
<th>Singleton</th>
<th>Twins (MC/DC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA: 10 to 32 weeks</td>
<td>TA: 10 to 32 weeks</td>
<td></td>
</tr>
<tr>
<td>TC: 10 to 11+6 weeks</td>
<td>TC: 10 to 11+6 weeks</td>
<td></td>
</tr>
</tbody>
</table>

**Risk of miscarriage:** above the estimated background rate or as the loss rate (total or at a specific GA beyond procedure-related effects) related to maternal age, GA at procedure, indication for procedure, provider experience

Estimated added post-procedure loss rate is 0.5% to 1.0% or total spontaneous and procedure loss rate is 1.9% to 2.0%

Estimated added risk\(^\text{57}\):

Total fetal loss rate for TA CVS = second trimester amniocentesis rate RR 0.9 (95% CI 0.66 to 1.23):

- TA: 1% to 2%
- TC: 2% to 6%

TC increased fetal loss by OR 1.40 (95% CI: 1.09 to 1.81).

Background spontaneous pregnancy and fetal loss rate is increased for twins.

Twin systematic review\(^\text{59}\) post procedure:

Total pregnancy loss: OR 3.84% (95% CI 2.48 to 5.47)

Total fetal loss: OR 5.48% (95% CI 4.06 to 7.13)

**Risk of congenital fetal disruptive anomaly**

Limb reduction < 9 weeks (66 days) (estimated at 1 in 3000) possible hemangioma

**Probability of successful procedure**

With a skilled provider > 99% with combination of both TC and TA techniques or approach

> 99% with combination of both TC and TA techniques and/or approach

**Time to laboratory diagnosis**

2 to 3 weeks (rapid direct FISH/PCR techniques can be used as required)

2 to 3 weeks (rapid direct FISH/PCR techniques can be used as required)

**Accuracy (chromosomes/aneuploidy/ translocation)**

Highly accurate for large chromosomal pathology

Highly accurate for large chromosomal pathology

**Other lab based testing**

| microarray | Yes | Yes |
| whole genome sequencing | Yes | Yes |

**Lab-based findings**

- **Mosaicism**
  - Confined to placenta; 1% to 2%
  - Confined to placenta; 1% to 2%

- **AFP**
  - No
  - No

- **ACHE**
  - No
  - No

- **Other**
  - Placenta-based genetic/biochemistry/enzyme
  - Placenta-based genetic/biochemistry/enzyme

**Other procedural risk**

There is no preeclampsia-induced or -associated risk with CVS. The first-trimester placental analytes result in screen positive results that require diagnostic testing by CVS.

\(^{57}\) RR: relative risk
### Table 7. Risk/benefit data for cordocentesis/PUBS

<table>
<thead>
<tr>
<th>Indications:</th>
<th>suspected fetal anemia; NAIT; NIH; aneuploidy; fetal Bg platelets; genetic analysis (mutation, biochemistry); fetal therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUBS is generally used for <strong>singleton</strong> fetal blood sampling only</td>
<td></td>
</tr>
<tr>
<td><strong>Gestational age range</strong></td>
<td>18 to 24 weeks</td>
</tr>
<tr>
<td><strong>Total risk of miscarriage</strong></td>
<td>18 to 24 weeks increased risk</td>
</tr>
<tr>
<td>No anomalies:</td>
<td>1%</td>
</tr>
<tr>
<td>Anomalies:</td>
<td>7%</td>
</tr>
<tr>
<td>IUGR:</td>
<td>14%</td>
</tr>
<tr>
<td>Hydrops:</td>
<td>25%</td>
</tr>
<tr>
<td><strong>Fetal anomaly disruptive risk</strong></td>
<td>Increased risk if sustained bleeding from cord with significant anemia and/or hypotension</td>
</tr>
<tr>
<td><strong>Probability of successful procedure (counselling points)</strong></td>
<td>With a skilled provider, greater than 98%</td>
</tr>
<tr>
<td>A small specimen can be confirmed in the lab to be fetal blood through testing for red blood cell MCV or Kleihauer-Betke criteria.</td>
<td></td>
</tr>
<tr>
<td>The best fetal vessel locations or sources the provider may choose from are the intra-hepatic vein, the fetal abdominal cord insertion site, the free cord loop, or fetal cardiac ventricle (right or left).</td>
<td></td>
</tr>
<tr>
<td><strong>Time to laboratory diagnosis</strong></td>
<td>Based on hematologic, biochemical, or genetic testing requested but similar to neonatal results</td>
</tr>
<tr>
<td><strong>Accuracy (chromosomes) aneuploidy/translocation</strong></td>
<td>Highly accurate for large chromosomal pathology</td>
</tr>
<tr>
<td><strong>Other lab-based testing</strong></td>
<td>Highly accurate: additive genetic information with standard normal karyotype(^{36})</td>
</tr>
<tr>
<td>With advanced maternal age and/or positive screen 1.7%</td>
<td></td>
</tr>
<tr>
<td>With structural anomaly 6.0%</td>
<td></td>
</tr>
<tr>
<td><strong>Lab-based findings</strong></td>
<td>Provides detailed genetic mutational information</td>
</tr>
<tr>
<td><strong>Mosaicism (bone marrow)</strong></td>
<td>Accurate but based on chromosomal mosaicism, %</td>
</tr>
<tr>
<td><strong>AFP</strong></td>
<td>Yes, if required</td>
</tr>
<tr>
<td><strong>AChE</strong></td>
<td>Yes, if required</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>any neonatal blood parameters</td>
</tr>
<tr>
<td><strong>Other procedural risks</strong></td>
<td>Umbilical cord bleeding 20% to 30%</td>
</tr>
<tr>
<td>Fetal bradycardia 5% to 10%</td>
<td></td>
</tr>
<tr>
<td>Vertical infection (hepatitis B or C; HIV) through maternal-to-fetal circulation Unknown, but estimated to be low</td>
<td></td>
</tr>
<tr>
<td><strong>Procedural protocol technical aspects</strong></td>
<td>Antibiotics</td>
</tr>
<tr>
<td>Maternal sedation</td>
<td></td>
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<tr>
<td>Local anaesthesia</td>
<td></td>
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<tr>
<td>Skin preparation</td>
<td></td>
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<tr>
<td>Needle guidance</td>
<td></td>
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<tr>
<td>Needle gauge and length</td>
<td></td>
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<tr>
<td>Paralytic agent</td>
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<tr>
<td>Sampling site</td>
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</tr>
</tbody>
</table>

NAIT: neonatal alloimmune thrombocytopenia; NIH: neonatal intraventricular hemorrhage; Bg: human leukocyte antigens class I; IUGR: intrauterine growth restriction; MCV: mean corpuscular volume
The data available for cordocentesis/PUBS is presented in Table 7. The introduction of non-invasive prenatal testing for fetal trisomy screening in low-risk (no added history or pregnancy-related risk) and high-risk (obstetrical screen positive or maternal age) populations will decrease the number of invasive procedures requested or required. This impact will be primarily on AC and CVS. This decrease in procedures will impact training and maintenance of skills for invasive procedure providers.58

**Recommendations**

2. The health care provider should counsel the at-risk pregnant woman with regards to the in utero genetic diagnosis technique(s) associated with the fetal genetic testing options, and review the risks/benefits as part of the informed consent process. (III-A)

3. During risk/benefit counselling, the health care provider should advise that the best estimate of the pregnancy loss rate related to:
   a. amniocentesis is 0.5% to 1.0% (range 0.17 to 1.53%) (I)
   b. chorion villus sampling is 0.5% to 1.0% (I) and
   c. cordocentesis or percutaneous umbilical blood sampling is 1.3% for fetuses with no anomalies and 1.3% to 25% for fetuses with single or multiple anomalies or intrauterine growth restriction. (II-2A)

**SUMMARY**

Risk/benefit counselling for in utero prenatal diagnosis procedures requires appropriate patient information with fetal-specific genetic depth of analysis and level of testing recommended to assist in the informed consent process.

Cost-effectiveness analysis (of medical, personal, and genetic information) are not yet available for these new prenatal diagnosis scenarios. Patient choice and consent will require new counselling processes and time commitments.

Prenatal in utero diagnostic procedures are considered to be relatively safe, but they do have a small added pregnancy loss risk over the natural or spontaneous pregnancy fetal loss rate.

The field is rapidly evolving, and SOGC, like other health organizations, will endeavour to stay abreast of evidence as it becomes available.

**REFERENCES**


