

# Prenatal Screening for and Diagnosis of Aneuploidy in Twin Pregnancies

This clinical practice guideline has been prepared by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists (CCMG) and approved by the Executive and Council of the Society of Obstetricians and Gynaecologists of Canada and the Board of Directors of the Canadian College of Medical Geneticists.

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## Abstract

**Objective:** To provide a Canadian consensus document with recommendations on prenatal screening for and diagnosis of fetal aneuploidy (e.g., Down syndrome and trisomy 18) in twin pregnancies.

**Options:** The process of prenatal screening and diagnosis in twin pregnancies is complex. This document reviews the options available to pregnant women and the challenges specific to screening and diagnosis in a twin pregnancy.

**Outcomes:** Clinicians will be better informed about the accuracy of different screening options in twin pregnancies and about techniques of invasive prenatal diagnosis in twins.

**Evidence:** PubMed and Cochrane Database were searched for relevant English and French language articles published between 1985 and 2010, using appropriate controlled vocabulary and key words (aneuploidy, Down syndrome, trisomy, prenatal screening, genetic health risk, genetic health surveillance, prenatal diagnosis, twin gestation). Results were restricted to systematic reviews, randomized controlled trials, and relevant observational studies. Searches were updated on a regular basis and incorporated in the guideline to August 2010. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology assessment-related agencies, clinical practice guideline collections, clinical trial registries, and national and international medical specialty societies. The

**Key Words:** Prenatal screening, twin pregnancy, prenatal diagnosis, amniocentesis, nuchal translucency, maternal serum screening, chorionic villus sampling

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previous Society of Obstetricians and Gynaecologists of Canada guidelines regarding prenatal screening were also reviewed in developing this clinical practice guideline.

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

**Benefits, harms, and costs:** There is a need for specific guidelines for prenatal screening and diagnosis in twins. These guidelines should assist health care providers in the approach to this aspect of prenatal care of women with twin pregnancies.

### Summary Statements

1. Fetal nuchal translucency combined with maternal age is an acceptable first trimester screening test for aneuploidies in twin pregnancies. (II-2)
2. First trimester serum screening combined with nuchal translucency may be considered in twin pregnancies. It provides some improvement over the performance of screening by nuchal translucency and maternal age by decreasing the false-positive rate. (II-3)
3. Integrated screening with nuchal translucency plus first and second trimester serum screening is an option in twin pregnancies. Further prospective studies are required in this area, since it has not been validated in prospective studies in twins. (III)
4. Non-directive counselling is essential when invasive testing is offered. (III)
5. When chorionic villus sampling is performed in non-monochorionic multiple pregnancies, a combination of transabdominal and transcervical approaches or a transabdominal only approach appears to provide the best results to minimize the likelihood of sampling errors. (II-2)

### Recommendations

1. All pregnant women in Canada, regardless of age, should be offered, through an informed counselling process, the option of a prenatal screening test for the most common clinically significant fetal aneuploidies. In addition, they should be offered a second trimester ultrasound for dating, assessment of fetal anatomy, and detection of multiples. (I-A)
2. Counselling must be non-directive and must respect a woman's right to accept or decline any or all of the testing or options offered at any point in the process. (III-A)
3. When non-invasive prenatal screening for aneuploidy is available, maternal age alone should not be an indication for invasive prenatal diagnosis in a twin pregnancy. (II-2A) If non-invasive prenatal screening is not available, invasive prenatal diagnosis in twins should be offered to women aged 35 and over. (II-2B)
4. Chorionicity has a major impact on the prenatal screening process and should be determined by ultrasound in the first trimester of all twin pregnancies. (II-2A)

### ABBREVIATIONS

AFP	alpha fetoprotein
CVS	chorionic villus sampling
DR	detection rate
FPR	false-positive rate
hCG	human chorionic gonadotropin
NT	nuchal translucency
PAPP-A	pregnancy-associated plasma protein A
uE3	unconjugated estriol

5. When screening is done by nuchal translucency and maternal age, a pregnancy-specific risk should be calculated in monozygotic twins. In dichorionic twins, a fetus-specific risk should be calculated. (II-3C)
6. During amniocentesis, both amniotic sacs should be sampled in monozygotic twin pregnancies, unless monozygosity is confirmed before 14 weeks and the fetuses appear concordant for growth and anatomy. (II-2B)
7. Prior to invasive testing or in the context of twins discordant for an abnormality, selective reduction should be discussed and made available to those requesting the procedure after appropriate counselling. (III-B)
8. Monitoring for disseminated intravascular coagulopathy is not indicated in dichorionic twin pregnancies undergoing selective reduction. (II-2B)

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## OVERVIEW OF TWIN PREGNANCIES: EPIDEMIOLOGY, ZYGOSITY, AND CHORIONICITY

### Epidemiology

In Canada and other developed countries, the incidence of multiple pregnancies has increased dramatically since the 1980s. Over the period 1995 to 2004, the rate of multiple births showed a steady increase from 2.2% to 3.0%.<sup>1</sup> The two major reasons for this increase are the rising maternal age because of delayed childbearing, and the increasing use of assisted reproductive technologies and ovulation induction.

### Zygoty and Chorionicity

Zygoty refers to the genetic identity of each twin in the pregnancy, and chorionicity relates to its placentation. In monozygotic twins, a single fertilized oocyte splits into 2 distinct individuals after a variable number of divisions. Such twins are almost always genetically identical and therefore of the same sex. On rare occasions, mutations or chromosomal non-disjunction cause genetic discordance, resulting in phenotypic and chromosomal differences between monozygotic twins.<sup>2</sup> When 2 separate oocytes are fertilized, dizygotic twins result. These individuals are genetically distinct and usually discordant for chromosomal anomalies. The incidence of spontaneous monozygotic twins is remarkably stable worldwide at approximately 4 per 1000 births. It is now clear that infertility treatments are associated with a 2- to 12-fold increased risk of monozygotic twinning.<sup>3–5</sup> The exact mechanisms of this association remain elusive.<sup>2</sup> The incidence of dizygotic twinning varies greatly with factors such as maternal age, ethnicity, and infertility treatments, sharing a common mechanism of increased FSH maternal serum levels. Overall, about 66% of twin pregnancies are dizygotic and 33% are monozygotic.

The determination of chorionicity of a twin pregnancy is of paramount importance, and it should ideally be assessed in the first trimester,<sup>6</sup> when its accuracy is 96% to

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

Quality of evidence assessment*	Classification of recommendations†
I: Evidence obtained from at least one properly randomized controlled trial	A. There is good evidence to recommend the clinical preventive action
II-1: Evidence from well-designed controlled trials without randomization	B. There is fair evidence to recommend the clinical preventive action
II-2: Evidence from well-designed cohort (prospective or retrospective) or case-control studies, preferably from more than one centre or research group	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
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	D. There is fair evidence to recommend against the clinical preventive action
III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees	E. There is good evidence to recommend against the clinical preventive action
	L. There is insufficient evidence (in quantity or quality) to make a recommendation; however, other factors may influence decision-making

\* 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.<sup>91</sup>

† Recommendations included in these guidelines have been adapted from the Classification of Recommendations criteria described in the The Canadian Task Force on Preventive Health Care.<sup>91</sup>

100% versus approximately 80% in the second trimester.<sup>7-9</sup> Chorionicity, rather than zygosity, has a major impact on the outcome of twin pregnancies,<sup>10,11</sup> mainly because of specific complications secondary to placental anastomoses, such as twin-to-twin transfusion syndrome. The description of these complications is beyond the scope of this guideline. The relationship between chorionicity and zygosity can sometimes be misleading for clinicians. All dizygotic pregnancies are dichorionic, whereas monozygotic pregnancies can be dichorionic (in approximately 33% of cases) or monochorionic (in 66% of cases), depending on the timing of embryo splitting.<sup>2</sup> Chorionicity can be determined by ultrasound, but zygosity cannot always be inferred from the determination of chorionicity. A monochorionic pregnancy is always monozygotic, with very rare exceptions that are due to post-zygotic changes.<sup>2</sup> Dichorionic twins with discordant sex are always dizygotic. In the case of same-sex dichorionic twins, zygosity cannot be determined with confidence unless genetic testing is performed (using microsatellites or single nucleotide polymorphism techniques).<sup>12</sup>

## PRENATAL SCREENING IN TWINS

### Aneuploidy Risk Estimation in Relation to Maternal Age, Zygosity, and Chorionicity

Overall, twin pregnancies are at higher risk than singleton pregnancies for aneuploidy. This is mostly due to advanced maternal age in twin pregnancies. Monochorionic twins carry a higher risk of structural abnormalities, including

cardiac defects, neural tube and brain defects, facial clefts, and gastrointestinal and anterior abdominal wall defects, but the risk of aneuploidy appears similar to the risk in singleton pregnancies. Down syndrome risk adjustment in twin or multiple pregnancies is complicated, since it poses complex practical and ethical issues. Zygosity, rather than chorionicity, determines the degree of risk and whether or not the fetuses may be concordant or discordant for chromosomal anomalies. In monozygotic pregnancies, both twins are either affected or unaffected, with very rare exceptions, while in dizygotic pregnancies, the risk of aneuploidy for each twin is more or less independent of the risk for the other. Modification of the maternal age threshold at which invasive testing is traditionally recommended in twin pregnancies has been suggested. Rodis et al.<sup>13</sup> proposed formulas to calculate the probability of aneuploidy in one or both dizygotic twins and in monozygotic twins. Charts derived from these calculations were used to determine age-related risk of fetal aneuploidy. The monozygotic twin rate was fixed at 3.5 per 1000 births. The authors determined that a 31-year-old woman with twins of unknown zygosity had the same risk of fetal aneuploidy as a 35-year-old woman with a singleton. They concluded that invasive prenatal diagnosis should be offered to all women  $\geq 31$  years with a twin pregnancy. This approximation should no longer be used for at least 4 reasons.

1. The procedure-related risk of invasive testing in twins is higher than in singletons, and the traditional thresholds used in singletons should be weighed against the fetal loss rate of invasive procedures.

2. This approximation is not valid in monochorionic pregnancies, which carry the same risk of aneuploidy as singleton pregnancies. The monozygotic rate is not fixed in twins, and it is extremely difficult to determine, especially when the pregnancy is a result of assisted reproduction techniques.
3. The fixed cut-off of 35 years for offering invasive testing is no longer recommended by Canadian guidelines in singleton pregnancies.<sup>14</sup>
4. The observed prevalence of Down syndrome in twin pregnancies is much less than the theoretical calculations predict<sup>15</sup> and is not simply double the prevalence in singletons, even in dizygotic pregnancies. Unfortunately, there are no large studies available to give a precise estimate of the incidence of Down syndrome in dizygotic twins, and most studies are based on statistical modelling.

### Recommendations

1. All pregnant women in Canada, regardless of age, should be offered, through an informed counselling process, the option of a prenatal screening test for the most common clinically significant fetal aneuploidies. In addition, they should be offered a second trimester ultrasound for dating, assessment of fetal anatomy, and detection of multiples. (I-A)
2. Counselling must be non-directive and must respect a woman's right to accept or decline any or all of the testing or options offered at any point in the process. (III-A)
3. When non-invasive prenatal screening for aneuploidy is available, maternal age alone should not be an indication for invasive prenatal diagnosis in a twin pregnancy. (II-2A) If non-invasive prenatal screening is not available, invasive prenatal diagnosis in twins should be offered to women aged 35 and over. (II-2B)

### Nuchal Translucency in Twins

Because the nuchal translucency distribution does not differ significantly between twins and singletons, the Down syndrome detection rate in multiples is similar to that of singletons.<sup>16–18</sup> In addition, NT can be determined separately for each twin. Therefore, NT measurement combined with maternal age has been the method of choice for prenatal aneuploidy screening in twins.<sup>14</sup> However, chorionicity and zygosity can both affect the estimated risk of Down syndrome in twins. Risk assessment can be calculated for each twin or for both together. In monochorionic twins, each fetus has the same risk of being affected with Down syndrome, and the overall risk is the same as in a singleton

pregnancy. Thus, the NT measurements are averaged to calculate a single risk estimate for the entire pregnancy using published NT values for singletons.<sup>18</sup>

Each fetus in a dichorionic twin pregnancy is considered separately, and the risk for each fetus is calculated by using median NT values for singletons.<sup>18</sup> The crown–rump length of each fetus is used to calculate gestational age. Since each fetus has an independent risk, risk calculation in dichorionic twin gestations can be done as a fetus-specific risk or a pregnancy-specific risk. In both circumstances, the NT and crown–rump length of each fetus is factored into the calculation. Although approximately 10% of dichorionic twins are actually monozygotic and should have their risk calculated as such, this small percentage has not been found to affect overall screening accuracy in this population.<sup>19</sup> However, this approach will likely increase the false-positive rate in monozygotic dichorionic twins. It is appropriate to calculate pregnancy-specific risk, because if a screen-positive woman with twins chooses to have invasive prenatal tests performed to obtain a diagnosis, it is standard clinical practice to test both dichorionic twins. Nevertheless, this pregnancy-specific risk has to be balanced with the risk of invasive procedures in twins rather than singletons, which requires using a specific threshold for screening in twins.

Using first trimester NT and maternal age, Sebire and colleagues calculated the specific risk for Down syndrome for each twin from 448 twin pregnancies.<sup>18</sup> The DR was 88% for a 7.3% FPR. Importantly, the prevalence of increased NT was higher in women with monochorionic pregnancies than in those with dichorionic pregnancies (8.4% vs. 5.4%), suggesting that increased NT in monochorionic twins may be an early manifestation of the twin–twin transfusion syndrome.<sup>20,21</sup> Therefore, the aneuploidy risk calculated by NT should be adapted in monochorionic twins. In a study of 769 monochorionic twins, Vandecruys et al.<sup>22</sup> found that the best screening performance was achieved using the average NT rather than the highest or lowest NT measured within a twin pair.<sup>22</sup> Using the average NT in monochorionic twins resulted in an estimated 100% sensitivity for a 4.2% FPR, and this is currently the method recommended by the Fetal Medicine Foundation in the United Kingdom.<sup>23</sup> In dichorionic pregnancies, the DR and FPR per fetus are similar to those in singleton pregnancies, and a fetus-specific risk is calculated. The results of screening by nuchal translucency and maternal age are summarized in Table 2. It would appear that NT in conjunction with maternal age in twin pregnancies has the potential to reach

**Table 2. Performance of screening by first trimester nuchal translucency and maternal age in twins**

Reference	Term risk cut-off	N (total twin pregnancies/ fetuses with T21)	DR for trisomy 21, %	FPR, %
Sebire et al., 1996 <sup>18</sup>	1:300	448/8	88	5.0 (fet), 10 (preg)-DC 13 (preg)-MC
Spencer, 2000 <sup>41</sup>		159/NA*	75.2	5.0
Maymon et al., 2001 <sup>42</sup>		174/2	100	9.0
Spencer & Nicolaides, 2003 <sup>27</sup>	1:300	230/4	75	6.8 (fet), 9.2 (preg)
Wald et al., 2003 <sup>19</sup>	NA*	NA*	73 (MC) 68 (DC) 69 (all twins)	
Vandecruys et al., 2005 <sup>22</sup>	1:300	769/6	100	4.2 (MC only—average NT)
Gonce et al., 2005 <sup>25</sup>	1:250	100/3	100	8.6 (fet), 14.3 (preg)
Chasen et al., 2007 <sup>24</sup>	1:130	535/7	83	5.0
	1:300		100	11.2

\* modelled data

DC: dichorionic; fet: risk calculated for each twin; preg: risk calculated for the entire pregnancy; NA: not available; MC: monochorionic.

the standard established in the SOGC 2007 guideline<sup>14</sup> of 75% sensitivity for a 5% screen positive rate; however, the large studies needed to confirm these numbers have not yet been published.

### Summary Statement

1. Fetal nuchal translucency combined with maternal age is an acceptable first trimester screening test for aneuploidies in twin pregnancies. (II-2)

### Recommendations

4. Chorionicity has a major impact on the prenatal screening process and should be determined by ultrasound in the first trimester of all twin pregnancies. (II-2A)
5. When screening is done by nuchal translucency and maternal age, a pregnancy-specific risk should be calculated in monochorionic twins. In dichorionic twins, a fetus-specific risk should be calculated. (II-3C)

### Nuchal Translucency Combined With Serum Markers

Few studies have examined the performance of first trimester serum screening using free  $\beta$ -hCG and PAPP-A in twins.<sup>19,24–27</sup> As in the second trimester, maternal serum markers are approximately twice as high in twin pregnancies as in singletons during the first trimester.<sup>16</sup> In a recent study of 1914 sets of twins, Spencer et al. examined the impact of chorionicity on first trimester serum markers.<sup>26</sup> They concluded that screening in twins requires adjustment of the calculated multiples of the median to account for the presence of 2 fetuses. In general, for free

$\beta$ -hCG, this should be by dividing the observed corrected multiples of the median by 2.023. For PAPP-A, 2 different factors are required: 2.192 in dichorionic twins and 1.788 in monochorionic twins. Spencer and Nicolaides reported a 75% DR of Down syndrome for a 9% FPR using NT and first trimester serum markers in 206 twin pregnancies, including 4 twins discordant for Down syndrome.<sup>27</sup> The DR by nuchal translucency and maternal age alone was similar. Wald et al.<sup>19</sup> published estimates of the screening performance of NT and combined screening for monochorionic, dichorionic, and all twins using “pseudo-risk” estimates. A true risk can not be calculated in twin pregnancies, because the distribution of the serum markers in twin pregnancies with Down syndrome and the distribution of NT values in affected fetuses from twin pregnancies are not known. For that reason a pseudo-risk is calculated, which consists of dividing the marker levels by the median level in unaffected twin pregnancies and thereafter interpreting them as if they had come from a singleton pregnancy. In dichorionic twin gestations, an unaffected twin can mask the presence of an affected twin when this calculation is used.<sup>19</sup> Wald et al.<sup>19</sup> found that for a fixed 5% FPR, the detection rates of nuchal translucency alone or in combination with first trimester serum markers were respectively 73% and 84% in monochorionic pregnancies, 68% and 70% in dichorionic pregnancies, and 69% and 72% in all twin pregnancies. The combination of NT and first trimester biochemistry may provide detection rates in twins similar to those in singletons, especially in monochorionic pregnancies. However, larger prospective studies on first trimester combined screening in multiples are needed before definitive recommendations can be made.

**Table 3. Performance of combined first trimester screening in twins: PAPP-A+free  $\beta$ -hCG + nuchal translucency + maternal age**

Reference	Term risk cut-off	N (total twin pregnancies/ fetuses with T21)	DR for trisomy 21, %	FPR, %
Spencer, 2000 <sup>41</sup>	NA	159/NA	80.1 (all) 79.7 (disc) 81.3 (conc)	5.0
Chasen et al., 2007 <sup>24</sup>	1:198	535/7	100	5.0
	1:300		100	7.0
Spencer & Nicolaides, 2003 <sup>27</sup>	1:300	206/4	75	NA
Sebire et al., 2000 <sup>21</sup>			73 (MZ); 43 (DZ)	5.0
Vandercruys et al., 2005 <sup>22</sup>	1:300	769/6	70 (disc); 84 (conc); 72 (all)	5.0
Wald & Rish, 2005 <sup>32</sup>	NA*	NA*	84 (MC)	5.0
			70 (DC)	5.0
			72 (all twins)	5.0

\* modelled data

NA: not available; disc: discordant for T21; conc: concordant for T21; MZ: monozygotic; DZ: dizygotic; DC: dichorionic; MC: monochorionic.

Studies combining first and second trimester maternal serum screening with first trimester NT determination in twins are also needed. Like maternal serum screening in the second trimester, serum screening of multiples in the first trimester has been criticized because abnormal serum levels from an affected twin will be brought closer to the mean by the unaffected twin. In addition, the effect of assisted conception on first trimester serum markers needs to be further assessed. The results of different studies on first trimester screening in twins are summarized in Table 3.

### Summary Statement

2. First trimester serum screening combined with nuchal translucency may be considered in twin pregnancies. It provides some improvement over the performance of screening by nuchal translucency and maternal age by decreasing the false-positive rate. (II-3)

### Maternal Serum Screening

The use of maternal serum screening in twin pregnancies raises a number of complications and unresolved issues. First, serum marker levels in twins are approximately twice those found in singleton pregnancies, but there are wide variations across studies because the numbers of cases and controls available are much smaller than for singletons.<sup>15,28–33</sup> As a result, the distribution of serum markers in twin pregnancies with Down syndrome is not known with reliability and pseudo-risks are estimated rather than based on large cohorts.<sup>32</sup> Second, the interpretation of the markers necessarily relates to the entire pregnancy,

while ultrasound markers such as NT are specific to each twin. Third, the role of zygosity and chorionicity in the risk estimation is similar to their role in NT screening.

In a prospective study of second trimester maternal serum screening in 274 twin pregnancies, the FPR was 5%.<sup>34</sup> As there were no cases of Down syndrome in the study population, the evaluation of sensitivity was not possible. Using statistical modelling, the authors estimated that the DR should be 73% in monozygotic twins and 43% in dizygotic twins, with an overall DR of 53% if a 5% FPR was maintained. In another study of second trimester screening in multiples, Spencer et al. evaluated free  $\beta$ -hCG and AFP in 420 twin and 19 triplet pregnancies.<sup>35</sup> On average, the markers were twice as high in the twin pregnancies. Eight sets of twins were found to be discordant for Down syndrome. The authors estimated that, using the pseudo-risk approach, the Down syndrome detection rate in twins should be 51% at a 5% FPR. Muller et al. evaluated second trimester maternal serum screening for Down syndrome in 3292 twin pregnancies.<sup>30</sup> Pregnancy outcomes were available in 3043 cases. Maternal serum AFP and free  $\beta$ -hCG were evaluated. In 4 pregnancies both twins had Down syndrome, while in 7 others only 1 twin was affected. Whereas median AFP levels were similar between dichorionic and monochorionic twins, free  $\beta$ -hCG levels were higher in monochorionic pregnancies. Down syndrome detection rates and screen positive rates were, respectively, 27.3% and 6.6% using maternal age alone, 54.5% and 24.6% using maternal age corrected for chorionicity, 54.5% and 7.75% using observed AFP and free  $\beta$ -hCG divided by 2, 54.5%

**Table 4. Performance of second trimester maternal serum screening in twins**

Reference	Term risk cut-off	N (total twin pregnancies/ fetuses with T21)	DR, %	FPR, %
Muller et al., 2003 <sup>30</sup> (dual)	1:250	3043/15	54.5	8.0 7.75 (MoM adjusted for chorionicity)
Garchet-Beaudron et al., 2008 <sup>29</sup> (dual)	1:250	11040/34	63 (all) 71 (conc) 60 (disc)	10.8
Maymon et al., 1999 <sup>36</sup>	1:380	60/1	100 (triple)	15.0
Cuckle, 1998 <sup>15</sup>	NA*	NA*	41 (dual) 44 (triple) 47 (quad)	5.0 5.0 5.0
Wald & Rish, 2005 <sup>32</sup> (integrated)	NA*	NA*	93 (MC) 78 (DC) 80 (all)	5.0

disc: discordant for T21; conc: concordant for T21; NA: not available; DC: dichorionic; MC: monochorionic.

\* modelled data; dual: AFP + hCG + maternal age (2nd trimester); triple: AFP + hCG + uE3 + maternal age (2nd trimester); quad: Total  $\beta$ -hCG + AFP + uE3 + inhibin A + maternal age (2nd trimester); integrated test: nuchal translucency + PAPP-A (at 10 to 13 wks) + AFP + uE3 + free  $\beta$ -hCG + inhibin A (at 14 to 22 wks) + maternal age.

and 8.05% using median values observed from the global twin population, and 54.5% and 7.75% using median values specific to monochorionic and dichorionic twins. The authors concluded that second trimester Down syndrome screening in twins is feasible and better than screening based on maternal age alone. More recently, in the largest series of second trimester serum screening (dual screening, using AFP and  $\beta$ -hCG) in twins reported to date, the same French group studied 11 040 twin pregnancies, including 27 pregnancies that were affected by Down syndrome.<sup>29</sup> The control group consisted of 64 815 singleton pregnancies, of which 86 were affected. Using a 1/250 cut-off, the overall Down syndrome DR in twin pregnancies was 63% (17/27) (95% CI 44.8% to 81.2%). Of twin pregnancies, 30.3% were in women over 35 years. When both twins were affected, detection rate was 71%, and when only one was affected, detection rate was 60%. In singleton pregnancies, DR was 74.4% (64/86) (95% CI 65.2% to 83.6%). The FPR in twins was 10.8%.

In another study, Maymon et al.<sup>36</sup> compared first trimester NT measurements with second trimester triple screening (AFP,  $\beta$ -hCG, and uE3) in 60 twin and 120 singleton pregnancies.<sup>36</sup> In the twins, the screen positive rate was lower with NT than with the triple screen (5% vs. 15%). In the singletons, the screen positive rate was also lower with NT than with the triple screen (2.5% vs. 6%). This high false-positive rate in the second trimester serum screening for twins led to an 18.3% amniocentesis rate in the twin group compared with

a 7.5% rate in the singleton group. Thus, in patients with twins, second trimester maternal serum screening may lead to higher rates of invasive prenatal diagnosis and associated fetal loss. As a result, it has been suggested that first trimester NT combined with maternal age may be the optimal way to assess Down syndrome risk in patients with a multiple pregnancy.<sup>14</sup> However, if NT screening is not available or has been missed because of the late diagnosis of a twin pregnancy (after 14 weeks), second trimester maternal serum screening may be considered in twins.

### Integrated Screening

In singleton pregnancies, integrated testing has been proposed to combine the benefits of first and second trimester screening.<sup>37</sup> The method, combining nuchal translucency and serum markers in the first and second trimesters, has been validated in singleton pregnancies in large prospective studies, showing high detection rates and low false-positive rates.<sup>38</sup> To date there are no prospective studies of the performance of integrated screening in twins. Wald and Rish have published estimations of the screening performance of integrated testing in twins.<sup>32</sup> Basing their calculations on a number of assumptions, they estimated that for a fixed FPR of 5%, the detection rate would be 93% in monochorionic twins, 78% in dichorionic twins, and 80% in all twins (Table 4). The estimated detection rate of the “serum integrated screening” without nuchal translucency is not available.

**Table 5. Counselling tips**

Different options are available for prenatal aneuploidy screening in twin pregnancies. To date, there are insufficient data to recommend a specific method of screening. Acceptable options are (1) screening by first trimester nuchal translucency and maternal age; (2) first trimester nuchal translucency and maternal age combined with serum biochemistry; (3) when nuchal translucency is not available, second trimester serum screening combined with maternal age.

Second trimester serum screening in twins leads to a high false-positive rate (about 10%) for a detection rate of 50% to 70%, depending on the number of markers used and the chorionicity. This method of screening in twins, although preferable to screening by maternal age alone, should be limited to cases where nuchal translucency is not available.

At present, there is insufficient evidence to make any recommendation about the use of second trimester genetic sonogram (soft markers) in twins for aneuploidy screening.

**Summary Statement**

3. Integrated screening with nuchal translucency plus first and second trimester serum screening is an option in twin pregnancies. Further prospective studies are required in this area, since it has not been validated in prospective studies in twins. (III)

**Second Trimester Ultrasound Screening for Aneuploidy in Twins**

Although the use of the genetic sonogram to detect Down syndrome in the second trimester has been well studied in singleton pregnancies, there are very few data to estimate the accuracy of this approach in twins.<sup>39</sup> Typically, the data in twin studies are combined with singleton data, and extrapolation of efficacy specific to twins is impossible. In one study, soft marker discordance was examined in twin sets discordant for Down syndrome. Nuchal thickness was found to correctly identify 5 of 9 instances of Down syndrome; the other markers were significantly less efficacious.<sup>40</sup> Currently, the data are insufficient to recommend for or against the use of ultrasound soft markers for aneuploidy in twins. Further prospective studies are needed to assess these markers in twins.<sup>6</sup>

**INVASIVE PRENATAL DIAGNOSIS FOR TWIN PREGNANCIES****Offering Invasive Testing in Multiple Pregnancies**

Non-directive counselling is required for patients with multiple pregnancies who are offered invasive testing (Table 5). Attitudes and choices of couples will vary greatly, and they may be different for couples whose twin pregnancies result from the use of assisted reproductive technologies after infertility and those with twin pregnancies conceived spontaneously.<sup>43</sup> The discussion should include a review of the options and the risks and benefits of each option available. The background risk of spontaneous pregnancy loss in twins (6% to 7%)<sup>44</sup> should be included when options are reviewed with couples. In all twin cases, but especially in dichorionic twin pregnancies, the possibility of discordant karyotypes and options available in that situation should also be reviewed.<sup>45</sup>

**Summary Statement**

4. Non-directive counselling is essential when invasive testing is offered. (III)

**Elements Common to All Invasive Diagnostic Procedures in Multiple Pregnancies**

A thorough sonographic assessment is mandatory before invasive prenatal testing is undertaken in multiple pregnancies. If chorionicity has not already been determined, attempts should be made to do so in view of the implications for screening (as described above) and for the management of abnormal results (concordant and discordant). Each twin's presentation and location must then be properly identified and labelled. Location of the sacs, fetuses, and placentas and cord insertions, as well as fetal sex, need to be evaluated and clearly documented using text and/or diagrams.<sup>45</sup> This is important to ensure that the samples, and thus the results, are assigned to the correct twin. In the rare event that selective termination is requested, this information is critical to ensuring that the procedure is performed on the correct fetus.<sup>46</sup>

Assessment of zygosity by molecular genetic testing may be useful in complex multiple pregnancies (for example, unsure chorionicity and discordance for fetal anomalies) or when chorionicity cannot be clearly established by ultrasound. Confirming dizygosity suggests likely dichorionicity and allows the pregnancy to be managed accordingly.<sup>47</sup>

**AMNIOCENTESIS****Technical aspects**

Despite a lack of large studies specific to early amniocentesis in multiple pregnancies, amniocentesis is typically performed at or after 15 weeks' gestation because of the increased risk associated with the earlier procedure in singletons.<sup>48</sup> Three techniques have been described, but they have not been compared in randomized studies.

### Two-puncture technique

The most frequently described technique involves 2 consecutive punctures using 2 different needles and 2 different locations, one on each side of the intertwin membrane as noted on ultrasound. The risk of sampling the same amniotic sac twice was estimated at 1.8% in a recent study of 260 twin pregnancies in one Canadian centre where dye was not instilled in the first sac.<sup>49</sup> Correlation between fetal sex and karyotype results may facilitate the identification of sampling errors (i.e., when the results demonstrate karyotypes of the same sex but the ultrasound suggests different sexes).

Dyes have been used to identify amniotic fluid samples coming from the same sac. With this technique variation, after the first fluid sample is taken, a dye is injected into the sac from which it was taken. When the second fluid sample is taken, the presence of dye raises the suspicion that the fluid sample comes from the first sac, and the location of the puncture site can be modified until a non-dyed sample is obtained. Methylene blue was first used, but its association with small bowel atresia and fetal death makes its use contraindicated.<sup>50–54</sup> Indigo carmine has since been used, with no reported increase in congenital anomalies over the expected background risk,<sup>55,56</sup> but at least 4 cases of jejunal atresia in infants exposed to this dye in utero have been reported.<sup>53,56,57</sup> Although it is unclear if that is an incidence higher than found in the general population, most operators use dye to identify gestational sacs only when ultrasound visualization is poor or in the case of higher-order multiples.<sup>46,58,59</sup>

### One-puncture technique

The second amniocentesis technique is the single puncture technique in which the needle is inserted close to the intertwin membrane under ultrasound guidance.<sup>60–64</sup> A fluid sample is taken from the first sac, and then the needle is advanced with ultrasound guidance through the membrane into the second sac. The first 1 mL to 2 mL of amniotic fluid is discarded to decrease the risk of contamination from the first sac, and then a sample is taken from the second sac. Potential challenges with this technique include tenting leading to difficulties in entering the second sac, the potential for contamination with fluid from the first sac,<sup>58</sup> and the risk of creating iatrogenic monoamnicity.<sup>65</sup> Even though these concerns were not encountered in 2 studies providing information on the technical aspects of the procedure in a total of 77 cases, this technique has not gained widespread acceptance.<sup>58</sup>

### Simultaneous visualization technique

The third technique involves simultaneous insertion and sonographic visualization of one needle into each sac.<sup>66</sup> Despite the advantage of documenting sampling on each side of the membrane, this technique is seldom used because of the time required to achieve this and the lack of clearly documented clinical advantages.<sup>58</sup>

### Single or double sampling in monochorionic twins

There is much debate about whether single or double sampling is required in monochorionic twins. In view of the multiple case reports of monochorionic twins with discordant karyotypes<sup>67–68</sup> and the difficulty in assessing monochorionicity at later gestational ages,<sup>7–9,69</sup> many advocate sampling of both amniotic sacs. This is particularly useful when the twins are discordant for fetal anomalies, nuchal translucency assessment, or growth,<sup>58</sup> or when chorionicity was not assessed before 14 weeks' gestation.<sup>6</sup>

#### Recommendation

6. During amniocentesis, both amniotic sacs should be sampled in monochorionic twin pregnancies, unless monochorionicity is confirmed before 14 weeks and the fetuses appear concordant for growth and anatomy. (II-2B)

### Pregnancy or fetal loss rate

The post-procedure pregnancy or fetal loss rate associated with amniocentesis in twins must be compared with the background loss rate associated with twins, ideally taking into consideration the chorionicity, because of the higher spontaneous loss rate in monochorionic twin pregnancies.<sup>11</sup> After correcting for as many confounding factors as possible, the most recent studies report an attributable loss rate varying from 0.3% to 2.2%.<sup>70–73</sup> A Canadian retrospective cohort study published in 2006 estimated the overall risk of pregnancy loss before 24 weeks' gestation to be 1 in 64 or 1.6%.<sup>71</sup> When a twin pregnancy follows fetal reduction of a higher order multiple pregnancy, amniocentesis does not seem to carry a greater risk of pregnancy loss than exists in higher order multiple pregnancies that undergo fetal reduction but no amniocentesis (total loss rate 8.1% vs. 12.5%; no statistical difference).<sup>74–75</sup>

### Gestational age and timing of the procedure

Studies in singleton pregnancies indicate that amniocentesis should not be carried out before 15+0 weeks, because of the increased risk of congenital anomalies and pregnancy loss.<sup>48</sup> When a discordance of karyotype between the twins is likely, the option of late amniocentesis can be considered once perinatal mortality and morbidity for the unaffected twin is

**Table 6. Procedure tips**

Carefully documented positional labelling of each fetus must be performed prior to proceeding with invasive testing. Chorionicity should also be assessed and documented (if not already determined).

Patients should be made aware that the risk of sampling error (sampling the same sac twice) is up to 1.8% when amniocentesis in twins is performed by experienced providers. Correlation of karyotype results with fetal sex assessment on ultrasound is recommended.

When a double puncture technique is used, consideration should be given to the instillation of dye following the first puncture, especially when ultrasound visualization is poor, or in higher order multiples.

Methylene blue should not be used as a dye in view of the associated risks of fetal small bowel atresia and fetal death.

Indigo carmine appears to be the safest dye currently available but should be used with caution.

There is a lack of evidence to support the superiority of the single or double puncture technique or simultaneous visualization in twin pregnancies in terms of pregnancy loss and sampling errors. Those performing the procedure should choose the twin amniocentesis technique most familiar to them.

Patients should be made aware that the attributable loss rate associated with amniocentesis in twin pregnancies is estimated to be 1 in 64 or 1.6%.

All amniocentesis in multiple pregnancies should be performed after 15+0 weeks' gestation by a maternal fetal medicine specialist or an individual with expertise in the procedure because of the complexity associated with the detailed mapping and sampling of the fetuses.

Patients should be made aware that the overall loss rate associated with CVS in twin pregnancies is estimated to be 3% to 4.5%.

Patients should be made aware that the risk of sampling error or inaccurate sampling when CVS is performed in twin pregnancies is estimated to be 3% to 4%.

CVS in multiple pregnancies should be performed by maternal fetal medicine specialists experienced in the technique because of the complexity associated with the procedure.

Patients should be made aware that the overall loss rate associated with CVS and amniocentesis in twin pregnancies appear to be similar, although the attributable loss rate has not been clearly defined for CVS.

Both CVS and amniocentesis appear as acceptable options for prenatal diagnosis in multiple pregnancies. A discussion of the advantages and disadvantages of each should provide the information the patient requires to make an informed decision based on her perceived advantages.

When prenatal diagnosis detects an abnormality in one or both twins, a genetic counsellor, geneticist, or maternal fetal medicine specialist should provide non-directive and unbiased information about the condition to facilitate the patient's decision-making process.

significantly lower. Although the benefits are obvious for a healthy twin, care providers must take into account both the risks of preterm labour and delivery occurring before results are obtained or before selective reduction (if requested) can be performed, and the psychological problems associated with delaying the procedure. Weisz and Rodeck<sup>58</sup> suggest that amniocentesis be delayed only when fetal anomalies associated with an increased risk of aneuploidy are diagnosed between 22 and 28 weeks' gestation and when the risk of complications associated with the procedure, resulting in severe prematurity for both twins, may outweigh the benefits of obtaining a diagnosis.<sup>58</sup> In those situations, a referral to a maternal fetal medicine specialist is particularly recommended.

## **CHORIONIC VILLUS SAMPLING**

### **Technical aspects**

The complexity associated with CVS is significant increased in multiple pregnancies compared with singletons. This includes accurate intrauterine mapping of the placentas (location and implantation site) and their relationship to each fetus, as well as the accurate sampling within each placenta away from its margin.<sup>58</sup> The relative filling of the bladder should also be noted, as it may significantly change

the position of the uterus and thus the relative position of the placentas and fetuses.<sup>76</sup> Frequent ultrasound (weekly) in the time interval between the CVS and the availability of the results has been recommended by some experts to follow the position of each fetus.

Both transabdominal and transcervical approaches have been used to perform CVS in multiples, either as sole method or in combination.<sup>76-82</sup> It is obviously important to replace the needle or the transcervical instrument for each separate sampling. Casals et al.<sup>77</sup> have reported a need for follow-up amniocentesis for a variety of indications, including lack of diagnosis and uncertainty of results in 15 out of 38 cases where both placentas were sampled transcervically, although technique improvement was noted with increasing experience.<sup>77</sup> In monozygotic twins, a single sample is indicated despite the small risk of heterokaryotypic monozygotic pregnancies.

### **Pregnancy or fetal loss rate**

The CVS post-procedural loss rate has been very difficult to establish. The number of samples needed to obtain an adequate specimen has been reported as 2.02 to 2.2, with up to 5 insertions being described.<sup>58</sup> The overall pregnancy loss rate for the group who underwent these procedures

appeared to be similar to the rate for a matched control group.<sup>58</sup> In a review of 4 studies with a total of 614 twin pregnancies undergoing CVS, the overall loss rate before 22 weeks was reported at 3.1%, with a total loss rate (up to delivery) of about 4.8%.<sup>78–82</sup>

### Chorionic villus sampling error

The risk of chorionic villus sampling error can be best assessed when concordant sex based on karyotypical analysis leads to the birth of discordant sex twins. Earlier experience reported a rate of up to 6% of such error in twin pregnancies undergoing CVS whereas more recent studies report lower rates of 2% to 4%.<sup>45,59,80,81,83</sup> Fluorescence in situ hybridization analysis identified fetal–fetal contamination more frequently (up to 11.5%).<sup>83</sup> In order to decrease the risk of sampling error, Jenkins and Wapner have suggested sampling near the placental cord insertion, avoiding the dividing membrane, and using a combined transabdominal and transcervical approach.<sup>45</sup> With these improvements, the current recommendation is to quote a risk of contamination or erroneous sampling around 3% to 4%.<sup>58</sup>

### Summary Statement

- When chorionic villus sampling is performed in non-monochorionic multiple pregnancies, a combination of transabdominal and transcervical approaches or a transabdominal only approach appears to provide the best results to minimize the likelihood of sampling errors. (II-2)

### Choosing Between CVS and Amniocentesis

The advantages of earlier diagnosis and access to earlier termination when CVS is performed may be even more relevant in the context of multiple pregnancies, as some authors have suggested that earlier selective terminations are associated with a lower risk of unintended pregnancy loss.<sup>84–85</sup> More recent data, however, do not seem to support this statement beyond 13 to 14 weeks of pregnancy. The use of CVS in the planning of multifetal reduction is beyond the scope of this guideline.

The technical challenges associated with the CVS procedure and the higher risk of sampling error favour amniocentesis. In a study comparing diagnostic accuracy between 286 amniocentesis procedures and 159 CVS procedures in twin pregnancies, van den Berg et al. reported successful sampling in 99.3% of amniocentesis and 99.7% of CVS with a 2-pass approach.<sup>86</sup> Results were inconclusive in 7 CVS (5 out of 7 that were due to placental mosaicism) and none of the amniocentesis procedures. Amniocentesis was used successfully to clarify the results in the 7 inconclusive CVS.

Only 2 studies are available to compare loss rates in amniocentesis and CVS in twin pregnancies.<sup>59,79</sup> They report

similar overall loss rates in twin pregnancy after CVS (3.2% to 4.5%) and amniocentesis (2.9% to 4.2%), and each study reports a non-significant difference of 0.3% in the overall loss rate, although neither was powered to assess such a difference. As these studies were not randomized but were contemporaneous cohorts, and as patient and provider choice determined the procedure selected, generalizations cannot be made and equivalence cannot be assumed. No procedure appeared to have a significant advantage when all were done by skilled and experienced operators.<sup>58,78</sup>

See Table 6 for a summary of clinical tips.

## MANAGEMENT OF TWINS DISCORDANT FOR KARYOTYPICAL ANOMALIES

### Initial Counselling

As in singleton pregnancies, patients facing a diagnosis of an abnormal fetal karyotype with or without associated anatomical anomalies in a twin pregnancy should receive the best information available in a non-directive and unbiased fashion that respects their values and acknowledges the needs of persons with disabilities and the varying perceptions of quality of life. Accurate information on the spectrum of short-term and long-term outcomes for the anomaly should be described and all questions answered.

### Selective Reduction

As discordance in twin karyotypes will usually occur in dizygotic and thus dichorionic twins, this guideline focuses on selective reduction in that context and does not review selective reduction in monozygotic twin pregnancies. Selective reduction should be performed only after the patient has been fully informed in a non-directive fashion about the anomaly and the procedure itself.

Decision-making may be challenging, and consultations with specialists in medical genetics and maternal fetal medicine are recommended to facilitate this. Counsellors with particular skills in decision-making support may also be useful to women and families facing these decisions.

### Recommendation

- Prior to invasive testing or in the context of twins discordant for an abnormality, selective reduction should be discussed and made available to those requesting the procedure after appropriate counselling. (III-B)

### Technical aspects

The most important imperative is to identify which twin has a chromosomal anomaly. This process starts at the time of the invasive procedure and must be carefully reassessed when

**Table 7. Selective reduction considerations**

Asystole of the selected twin should be confirmed for at least 5 minutes after the selective reduction is performed.

Patients contemplating a selective reduction should be made aware that the loss rate following a selective reduction is between 2.4% and 7.9% in dichorionic twin pregnancies and between 8.2% and 11.1% in triplets and higher order multiples. The rate of prematurity following the procedure is similar to that for dichorionic twins without selective reduction.

There is a need for further studies on the ideal timing of selective reduction in dizygotic twin pregnancies.

discordant karyotypes are identified. Detailed review of the descriptions at the time of the invasive testing and ultrasound assessment is required as soon as possible after the diagnosis is made.<sup>76</sup> Any diagnostic uncertainty should lead to reassessment by invasive testing and rapid karyotype determination.<sup>76</sup>

In dichorionic twin pregnancies, selective termination is typically performed transabdominally by inserting a 20- or 22-gauge needle in the fetal heart and injecting potassium chloride or lidocaine until asystole is confirmed. As some failures have been associated with confirmation of asystole for only 2 minutes, many experts recommend observation for at least 5 minutes, and some also recommend reconfirmation within 24 to 48 hours of the procedure.<sup>76</sup> The fetus remains within the uterus while the pregnancy continues and is typically delivered at the same time as the live twin. Delivery of the reduced twin days to weeks before the live twin has been described. In these cases, the reduced twin has been positioned lower in the uterus than the live twin. Lynch et al.<sup>87</sup> reported an odds ratio of preterm delivery of the live twin of 4.1 (95% CI 1.4 to 12.3) if the reduced twin was the presenting one.<sup>87</sup> Berkowitz et al.<sup>88</sup> described 3 cases of coagulation parameter change in their first 100 selective reductions in dichorionic twins, but none was clinically significant.

See Table 7 for a summary of considerations in selective reduction.

### Recommendation

8. Monitoring for disseminated intravascular coagulopathy is not indicated in dichorionic twin pregnancies undergoing selective reduction. (II-2B)

### Pregnancy outcome

Evans et al. described an international experience with 402 cases and an overall loss rate of 8.2% after selective reduction, with the highest loss rate being noted in women with 3 or more fetuses at the beginning of the pregnancy.<sup>89</sup> The overall loss rate following a selective reduction in twins was 7.9%. However, a study of 200 selective reductions in a single centre reported a loss rate in dichorionic twin pregnancies of 2.4% compared with 11.1% in pregnancies with 3 or 4 fetuses.<sup>90</sup>

In pregnancies without a loss, the average gestational age at delivery is 36 to 37 weeks, with the largest series reporting 6% of deliveries between 25 and 28 weeks.<sup>89,90</sup> These values are similar to outcomes in a dichorionic twin population, suggesting that the selective reduction itself has little effect on the prematurity rate.

### Timing of the procedure

Evans et al. reported a trend towards higher loss rate with increasing gestational age (from 5.4% at 9 to 12 weeks to 8.7% at 13 to 18 weeks and 6.8% at 19 to 24 weeks) but this was not statistically significant.<sup>89</sup> Eddleman et al. noted the opposite trend, with the loss rate for procedures before 20 weeks at 5.9% compared with 1.3% after 20 weeks.<sup>90</sup> Again, this difference was not statistically significant ( $P = 0.09$ ).

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