

5. Testing during pregnancy

Contents

5.1 Key points	2
Pre-pregnancy counselling.....	2
What are prenatal tests for birth defects	3
Risk factors.....	3
Offering testing	4
5.2 Background	7
Prenatal screening tests	7
Diagnostic testing	11
Detecting fetal anomalies	13
5.3 Frequently asked questions	15
5.4 Correcting misunderstandings	16
Appendix 1: FISH	17
Appendix 2: Trisomy 18	18
Appendix 3: Trisomy 13	19
Appendix 4: Pre-implantation genetic diagnosis	20
5.5 Patient and further information	21
5.6 Where to refer	22
5.7 References	23

Acknowledgements

We would like to thank the following people who contributed to the development of this section:

Dr Fiona Cullinane, Obstetrician (Royal Women's Hospital)
 Associate Professor Lachlan DeCrespigny, Obstetrical Ultrasonologist (Murdoch Children's Research Institute, Melbourne Ultrasound for Women)
 Dr Janet Duke, Obstetrician, Honorary Secretary (RANZCOG)
 Ms Robin Forbes, Coordinator, Maternal Serum Screening Program (Genetic Health Services Victoria)
 Dr Stephen Grigoleit, General Practitioner – Obstetrics (Moe Medical Centre)
 Dr Jane Gunn, General Practitioner and Senior Lecturer (The University of Melbourne)
 Dr Jane Halliday, Head, Epidemiology and Genetics Unit (Murdoch Childrens Research Institute),
 Manager of Victorian Birth Defects Register (Department of Human Services)
 Mrs Toni MacDonald, Support Services Representative (Down Syndrome Association of Victoria Inc)
 Dr Susan Nicolson, General Practitioner and GP Liaison Officer (Mercy Hospital for Women)
 Mr Mark Pertile, Head, Prenatal Cytogenetics Laboratory (Genetic Health Services Victoria)
 Dr Ewa Pjeiko, General Practitioner (Royal Australian College of General Practitioners)
 Dr Ines Rio, GP Liaison Officer (Royal Women's Hospital)
 Ms Ann Robertson, Genetic Counsellor (Human Genetics Society Australasia),
 Special Projects Officer (RANZCOG)
 Ms Jacinta Ryan, Senior Medical Scientist (Genetic Health Services Victoria)
 Dr Amanda Sampson, Clinical Director of Ultrasound (The Royal Women's Hospital)
 Associate Professor Les Sheffield, Clinical Geneticist and Director of Clinical Training
 (Genetic Health Services Victoria)
 Dr Susan White, Consultant Clinical Geneticist (Genetic Health Services Victoria)

5.1 Key Points

- Most babies are born healthy, but 4% of babies are born with a birth defect that may require medical care. Testing during pregnancy may identify some of these babies.
- Knowledge of a birth defect enables the parents to make decisions regarding the pregnancy or to plan for the future.
- **All couples should have the opportunity to consider testing.**
 - ✦ The best time to raise the issue of testing during pregnancy is before conception. GPs should discuss prenatal testing as part of pre-pregnancy counselling.
 - ✦ Discussion should include the possible outcomes, the decisions that the couple may face and their medical and psychosocial impact.
 - ✦ Testing should also be raised as soon as the pregnancy is confirmed. At this time, the greatest range of options is available. These options may be reduced by the time of the first antenatal visit.
- Some women choose not to have testing during pregnancy (Halliday et al. 2001). Tests should just be provided as options for couples and are not essential.

Pre-pregnancy counselling

Pre-pregnancy counselling allows potential risk factors to be identified and the risk of birth defects to be minimised through diet and lifestyle changes.

Pre-pregnancy counselling should include:

- Collection of relevant family history (see box below).
- Provision of information about screening and diagnostic tests during pregnancy.
- Folate supplementation.¹
- Thalassaemia screening.
- Drug and medication use and implications for pregnancy.
- Lifestyle changes: alcohol, smoking.

Information about infectious diseases:

- Rubella vaccination status
- Varicella antibody status and immunisation if non-immune.
- Discussion about Listeria infection² and Toxoplasmosis

Collecting the family history

The family history of both the woman and her partner should be collected regarding:

- Genetic conditions; for example, cystic fibrosis, fragile X syndrome, Duchenne muscular dystrophy.
- Down syndrome and other chromosome abnormalities.
- Birth defects; for example, spina bifida, cleft lip/palate, cardiac defects.
- Intellectual disability.
- Recurrent miscarriage.
- Unexplained perinatal deaths.
- Consanguinity.

¹ See Folate: a guide for health professionals, <http://www.dhs.vic.gov.au/phd/folate/hlthprof.htm>

² See Listeria: the facts', <http://www.dhs.vic.gov.au/phd/9907063/index.htm#3>

What are prenatal tests for birth defects?

- Prenatal tests include screening tests and diagnostic testing. See *Background* for specific information.

Screening tests

- *Nuchal translucency screening, combined first trimester screening and second trimester maternal serum screening.*
- Tests can determine who is at increased risk of having a baby with Down syndrome.
- Low-risk test results do not exclude Down syndrome or other chromosome abnormalities.
- Neural tube defects may also be detected with some tests.

See Table 1 for a summary of screening tests

Diagnostic testing

- Are performed using sampling procedures such as *amniocentesis* and *chorionic villus sampling*.
- Can diagnose Down syndrome, other chromosomal abnormalities and certain genetic conditions (indicated where there is a family history).

See Table 2 for a summary of diagnostic tests

Ultrasound scanning

- Ultrasound scans are screening tests for some birth defects (including chromosome abnormalities).
- Ultrasound scans can be a diagnostic tool for some birth defects (for example, neural tube defects).
- A second trimester ultrasound scan is not recommended as a primary screening test for Down syndrome (RANZCOG & HGSA 2001).

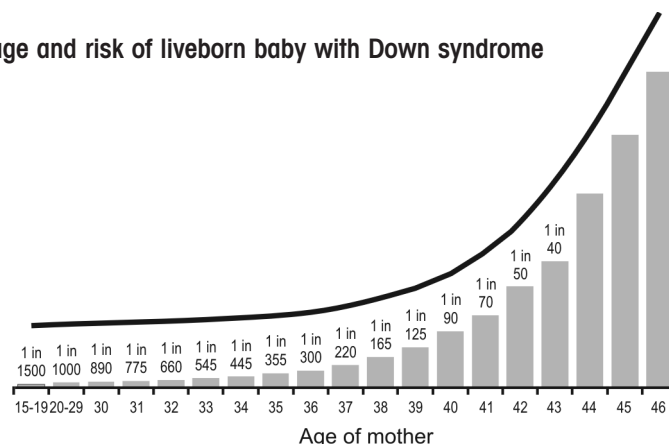
Ultrasound scanning for fetal anomaly should not be considered routine, but should be offered as a choice to women.

Risk factors

Down syndrome and other chromosomal abnormalities

- All women have a risk of having a baby with a chromosome abnormality, the most common being Down syndrome. See Part F *Down syndrome* for further information about this condition.
- The risk of Down syndrome depends on maternal age and increases with age. See Figure 1.

Figure 1. Maternal age and risk of liveborn baby with Down syndrome



Source: Gardner, RJ & Sutherland GR 1996, *Chromosome abnormalities and genetic counselling*, Oxford University Press.

- **Other factors that increase the risk of having a baby with Down syndrome include:**
 - ✦ Advanced maternal age.
 - ✦ A previous pregnancy with a chromosome trisomy.
 - ✦ An increased risk result on a screening test.
 - ✦ The presence of soft signs of Down syndrome during ultrasound examination.
 - ✦ A parent carrying a translocation involving chromosome 21 (see Part F *Down syndrome*).
- **Factors that may increase the risk of having a baby with other chromosome abnormalities:**
 - ✦ An increased risk result on a screening test.
 - ✦ The presence of soft signs or fetal anomalies during ultrasound examination.
 - ✦ A parent carrying a translocation.

Neural tube defects

The risk of a baby having a neural tube defect:

- Is approximately 0.1%, but is higher if there is a past or family history of the condition.
- Does not increase with maternal age.
- Is increased for women on anticonvulsants. Advice regarding risk is available from drug information services in obstetric hospitals.

Genetic conditions and birth defects

- A family history of a birth defect (for example, congenital heart defect), inborn error of metabolism (for example, PKU) or other genetic condition (for example, thalassaemia) may indicate an increased risk of that condition.
- A family history from the woman and her partner should be collected. If there are significant features (see box), *they should be referred to a genetics service for further risk assessment, preferably prior to conception.*

Offering testing

- All pregnant women or couples contemplating pregnancy should be offered information regarding screening tests. Women should be informed that they would be offered further testing if they have an increased risk result on a screening test.
- All women at increased risk should also be offered information about diagnostic tests. In Victoria women over 37 years at EDD are considered at 'increased risk'.
- If a woman decides to have prenatal testing, the best test will depend on the woman's gestation, risk and her concerns. Access to services may also influence the decision. The decision about which test is the best is a personal one for each woman/couple.
- Not all tests are available in the public sector and some tests do not have a Medicare rebate.
- Risk can be difficult for individuals to understand. Explaining risk is discussed in detail in the Part 1 Talking with families about genetics.

Table 1. Advantages and limitations of screening tests during pregnancy

Test	Gestation (weeks)	% of DS pregnancies detected ^a	Advantages	Disadvantages
Nuchal translucency screening (ultrasound) ^b	11 ³ –13 ⁶	75%	<ul style="list-style-type: none"> • Early screen and therefore early diagnosis • Detection of some fetal abnormalities • Benefits relating to early scan <ul style="list-style-type: none"> ✦ Accurate dating ✦ Diagnosis of multiple pregnancy ✦ Detection of early pregnancy failure ✦ Detection of some fetal abnormalities 	<ul style="list-style-type: none"> • Will detect some affected pregnancies that may spontaneously miscarry • May be limited access in Victoria • Out-of-pocket costs
Combined first trimester screening (ultrasound & blood test) ^b	10–12 (blood test) 11 ³ –13 ⁶ (ultrasound) ^b	85–90% (estimate only)		
Second trimester maternal serum screening (MSS)	14–20 (15–17 ideal)	70–75% ^c	<ul style="list-style-type: none"> • Available to women presenting in second trimester • Available throughout Victoria • No out-of-pocket costs for public patients if arranged the public hospital 	<ul style="list-style-type: none"> • Later screening test • Inaccurate dates can result in inaccurate risk by calculations. A dating scan should be considered if dates are uncertain

^a This is an average detection rate across all age groups. Detection rate increases with increasing maternal age.

^b Nuchal translucency measurement should be performed by a RANZCOG accredited operator.

^c Assumes use of the quadruple test (four analytes) and ultrasound dating.

Table 2. Advantages and limitations of diagnostic tests

Procedure	Gestation (weeks)	Advantages	Disadvantages
Chorionic villus sampling (CVS)	From 11 weeks ^a	Early detection Definitive diagnosis	Miscarriage risk (approximately 1% in expert hands) above background Detects chromosomally abnormal pregnancies that may spontaneously miscarry 1% risk of equivocal results (mosaicism) 0.1% failure to detect chromosome abnormality (abnormality is present in fetus but not in the placenta)
Amniocentesis	From 15 weeks ^a	Test with lowest miscarriage risk Definitive diagnosis	Miscarriage risk (approximately 0.5% in expert hands) above background Diagnosis in second trimester, when the pregnancy is more established

^a Note that women having procedures after 20 weeks gestation, may not receive diagnostic results in a time frame that permits termination of pregnancy.

5.2 Background

Prenatal screening tests

Introduction

- **Prenatal screening tests include:**
 - ✦ Nuchal translucency screening (ultrasound).
 - ✦ Combined first trimester screening (ultrasound and blood test).
 - ✦ Second trimester maternal serum screening (blood test).
- Screening tests determine who is at increased risk of having a baby with Down syndrome. Women choosing screening tests should be informed that they will be offered further testing if they have an increased risk and that they may choose to proceed to diagnostic testing. Reasons for not having diagnostic testing include concern about the risk of miscarriage, not wishing to know prior to the birth, and termination of pregnancy being unacceptable to the family.
- Screening tests are *non-invasive* so there is no risk of miscarriage from the procedure.
- Every screening test has a 'false positive' rate, where women receive an increased risk result but the baby is unaffected. This rate is usually set at approximately 5%.
- In the majority of pregnancies with an increased risk result on a screening test, the baby is unaffected and healthy. A common misconception held by women is that screening tests 'show' the fetus has Down syndrome. It can take some listening, clarification and explanation to counteract this belief. Anxiety at any increased risk result is normal (see Part A *Talking with families*).
- Low risk results do not exclude Down syndrome or other abnormalities.
- A second trimester ultrasound scan may detect some birth defects but is a relatively poor screen for Down syndrome.
- Neural tube defects and some other conditions may also be detected with some screening tests. The method with the highest detection rate for neural tube defects is an ultrasound scan during the second trimester (18–20 weeks).

The effect of maternal age on screening tests for Down syndrome

Screening tests give a *risk figure* for Down syndrome that is more specific than a risk based on age alone.

- The *detection rate depends on the type of test and the age of the woman*.
- The *detection rate and probability of an increased risk result increases with maternal age* as the calculations of risk will usually include the woman's age (Webley & Halliday 2002).
- For some women aged 37 years or older, screening tests are an acceptable alternative to diagnostic testing. It is important that the woman (couple) understands that screening tests will not identify all pregnancies with Down syndrome.

Nuchal translucency screening

Nuchal translucency screening uses nuchal translucency measurement in conjunction with gestation and maternal age to calculate the risk of Down syndrome by specialised computer software. The test may also detect pregnancies at increased risk for other chromosomal disorders, cardiac defects and some rare genetic conditions. The ultrasound is performed between *11 weeks 3 days and 13 weeks 6 days*.

Nuchal translucency measurement determines the thickness of fluid between the skin and soft tissue at the back of the fetal neck. This fluid appears as a translucent area on an ultrasound. Nuchal translucency measurement is highly operator dependent, requiring training, external quality control and adequate time to allow accurate measurement (Crossley et al. 2002). Measurement should be performed by a credentialed operator³ who is licensed using risk assessment software.

Figure 2. First trimester ultrasound scan. The crosses indicate nuchal translucency



Overall 75%⁴ of fetuses with Down syndrome are detected by nuchal translucency screening but the percentage is higher in older women (Sheffield 2002). Older women are also more likely to receive an increased risk result due to their age-related (prior) risk. The accuracy depends on operator experience and the use of risk-calculation software.

Nuchal translucency screening is an early screening test, which also has the benefits of an early scan including accurate dating, detection of multiple pregnancy, pregnancy failure or some fetal abnormalities. Disadvantages include cost, limited availability, and the early detection of affected pregnancies that may spontaneously miscarry.

Results are usually given at time of the test. Approximately 5% of tests give an increased risk (Sheffield 2002). Women with an increased risk are offered diagnostic tests. The majority of increased risk results are not due to Down syndrome and most of these babies will be healthy. The thicker the nuchal translucency in a chromosomally normal fetus, the greater the risk of structural anomalies and associated syndromes. These pregnancies may require closer monitoring.

See *Counselling for an increased risk screening result*.
See *Diagnostic testing*.

The possibility of false positive results and management options if risk is increased should be discussed with women prior to screening.

Arranging nuchal translucency screening

- Refer to a credentialed ultrasound operator with specialised risk assessment software.
- Nuchal translucency measurement and screening are not currently available in the public sector but may sometimes be performed as part of a first trimester scan for other indications, such as uncertain dates, multiple pregnancy or risk of miscarriage.
- Nuchal Translucency measurement and screening do not have Medicare item numbers and there are usually out-of-pocket costs.

³ Information about credentialed operators is available from RANZCOG (call (03) 9412 2939) or visit the website: <http://www.nuchaltrans.edu.au/>

⁴ Figure assumes that the operator is credentialed by RANZCOG and is applying the risk assessment software. Figure assumes that the operator is credentialed by RANZCOG and is applying the risk assessment software.

Combined first trimester screening

Combined first trimester screening uses a *blood test followed by nuchal translucency measurements* to give a combined risk figure of Down syndrome. The test may also detect pregnancies at increased risk for Trisomy 18 (Appendix 2).

The results are most accurate when blood is collected at 10 weeks gestation (Cuckle & van Lith 1999) but blood can be collected up to 12 weeks. The ultrasound scan is performed between 11 weeks 3 days and 13 weeks 6 days gestation. Blood should be collected prior to the ultrasound scan.

Two proteins present in maternal blood are measured. These are PAPP-A and β -hCG. Levels of these proteins vary, but tend to be different in women who are carrying fetuses with Down syndrome or trisomy 18. Increased β -hCG with decreased PAPP-A is suggestive of Down syndrome, while decreased levels of both analytes is suggestive of trisomy 18.

Up to 85 to 90% of fetuses⁵ with Down syndrome may be detected by combined first trimester screening (Sheffield 2002) but the percentage is higher in older women. The accuracy depends on ultrasound operator experience and the biochemical laboratory, the use of risk calculation software and the timing of the tests.

Combined first trimester screening is the screening test with apparently the highest detection rate for Down syndrome and the lowest rate of false positive results. It is an early screening test, which also has the benefits of an early scan including accurate dating, detection of multiple pregnancy, pregnancy failure and some fetal abnormalities. Disadvantages include cost, limited availability and the early detection of affected pregnancies that may spontaneously miscarry.

Results are available on the day of the ultrasound, if blood was collected prior to the ultrasound at 10 to 12 weeks gestation. Approximately 4% of tests give an increased risk. Women with an increased risk are offered diagnostic tests. The majority of increased risk results are not due to Down syndrome and most of these babies will be healthy. An abnormally thickened nuchal translucency in a chromosomally normal fetus indicates an increased risk of structural anomalies. These pregnancies may require closer monitoring.

See *Counselling for an increased risk screening result*.

See *Diagnostic testing*.

The possibility of false positive results and management options if risk is increased should be discussed with women prior to screening.

Arranging combined first trimester screening

- Tests should be arranged a couple of weeks in advance to allow time to coordinate the blood test and ultrasound. The blood test should be performed first.
- Arrange the ultrasound scan first with a credentialled operator for between 11 weeks 3 days and 13 weeks 6 days gestation.
- Arrange the blood collection for 10 to 12 weeks gestation by contacting Genetic Health Services Victoria Serum Screening program on (03) 8341 6303. Blood can also be collected at the local pathology service but the request form should have clear instructions for the sample to be sent to Genetic Health Services Victoria.
- The following information must be written on the blood test request form:
 - ✦ LMP & EDD
 - ✦ Current weight
 - ✦ Date and location of ultrasound scan.
 If the results of the scan are not received by the date on the form, the laboratory will contact the ultrasound practice or requesting doctor.
- First trimester screening is currently not available in the public sector. There are out of pocket costs for the blood test in addition to the cost of the ultrasound scan (see *Nuchal translucency screening*). Contact your service provider for details of test costs.

⁵ Local data are not yet available, as these are new services.

Second trimester maternal serum screening

Second trimester maternal serum screening uses a blood test in conjunction with the maternal age, gestational age and maternal weight to calculate a risk figure for Down syndrome. Second trimester maternal screening may also detect pregnancies at increased risk for trisomy 18 (see Appendix 3) and neural tube defects. Testing can be performed between 14 and 20 weeks but between 15 and 17 weeks is optimal.

The quadruple test measures four analytes (AFP, uE3, β -hCG and either α -hCG or inhibinA). The triple test measures three analytes. Testing is more accurate when four analytes are used.

Maternal blood contains substances produced by the fetus and placenta. The levels of alpha-fetoprotein (AFP), unconjugated estriol (uE3), the free b-subunit of human chorionic gonadotrophin (free β -hCG), α -hCG or inhibinA vary but, on average, their level will be altered in pregnancies affected by Down syndrome, trisomy 18 or neural tube defects. In Down syndrome, the levels of AFP and uE3 tend to be reduced, and α -hCG, β -hCG and inhibinA increased. In neural tube defects AFP may be increased and, in trisomy 18, levels of all of these substances are decreased.

Overall, approximately 70 to 75% (Crossley et al. 2002)⁶ of fetuses with Down syndrome are detected by maternal serum screening (Sheffield 2002) and approximately 5% of tests give an increased risk result. Women aged 40 and over have higher detection rates, with 95% of affected pregnancies receiving an increased risk result (Sheffield 2002). However at least 50% of all women in this age group will receive an increased risk result.

Results are available in approximately seven to 10 days. Women at increased risk are offered diagnostic testing. The majority of increased risk results are not due to Down syndrome and most of these babies are healthy.

See *Counselling for an increased risk screening result*.
See *Diagnostic testing*.

The possibility of false positive results and the management options should be discussed with women prior to screening.

Factors affecting the test result include:

- *Timing of the test.* If dates show that the woman was less than 14 weeks gestation at the time of testing, a blood recollection is necessary.
- *Accuracy of pregnancy dating.* When dates are based on LNMP alone, 30% of increased risk results are due to inaccurate dating. LMP or ultrasound dates are used to calculate the risk, and are most accurate. The risk should be recalculated if the gestational age is out by more than 14 days.
- *Weight.* All markers tend to be decreased in heavier women and increased in lighter women.
- *Insulin-dependent diabetes.* Levels of AFP and uE3 are lower in women with IDDM.
- *Twins.* All of the markers are raised in twin pregnancies. Occasionally the test will lead to the diagnosis of previously undiagnosed twins. It is difficult to interpret the test results for Down syndrome when twins are present.

Arranging second trimester maternal serum screening

- The number and type of analytes used vary between pathology services.
- The request form must include:
 - ✦ Ultrasound dates or LMP.
 - ✦ Weight.
 - ✦ If the woman has insulin-dependent diabetes.
 - ✦ Whether the woman is a share care patient and of which hospital.

⁶ This detection rate is for the quadruple test with ultrasound dating.

- There is no out-of-pocket cost to public patients if ordered by the hospital and performed through Genetic Health, or share care patients if tested through Genetic Health and request slip states 'share care patient'. Genetic Health receives government funding to perform testing on public patients.
- Some pathology services will forward samples to Genetic Health for testing.
- For private patients, the cost depends on the pathology provider.
- A second trimester maternal serum screening test does not need to be repeated unless blood was collected at less than 14 weeks gestation.

Counselling for an increased risk screening result

- It may be helpful to discuss the results with the service provider prior to informing the woman.
- Listen and give the woman (couple) time to absorb the news, consider her options and to make decisions about further testing. Informed decision making is assisted by the provision of up to date, unbiased information. See also Part F *Down syndrome* for information and further referral sources.
- Ensure the woman understands that she is part of a *group* of women with *increased* risk of the condition and that it is *not a definitive test result*.
- A common misconception held by women is that screening tests 'show' the fetus has Down syndrome. It can take some listening, clarification and explanation to counteract this belief. Anxiety at any increased risk result is normal (see Part A *Talking with families*).
- Reassure the woman that the majority of babies with an increased risk result will be *normal and healthy*.
- Discuss the option of diagnostic testing. Not all women decide to proceed to diagnostic testing. Reasons for not having diagnostic testing include concern about the risk of miscarriage, not wishing to know prior to the birth, and termination of pregnancy being unacceptable to the family.

Refer to the clinical genetics service at the woman's hospital. If there is not a service at the hospital, consider referral to Genetic Health Services Victoria (see *Where to refer*) for free post-test counselling. Consider referral for discussion of diagnostic tests and counselling for women with increased anxiety.

Diagnostic testing

Introduction

- Diagnostic testing requires invasive sampling procedures to obtain cells for chromosome analysis or specific genetic tests.
- **There are two types of procedures to obtain cells for chromosome analysis:**
 - ✦ Chorionic villus sampling
 - ✦ Amniocentesis.
- Both sampling procedures have a risk of miscarriage. The risk is operator dependent so tests should be performed by an experienced operator who performs the procedures frequently.
- **Indications for offering diagnostic testing include:**
 - ✦ Advanced maternal age (diagnostic testing can be performed in the public system for women aged 37 years and older).
 - ✦ A previous pregnancy with a chromosome trisomy.
 - ✦ The presence of soft signs of chromosome abnormality during ultrasound examination.
 - ✦ A parent carrying a chromosome translocation.
 - ✦ An increased risk result on a screening test.
 - ✦ An increased risk of having a baby with a genetic condition in specific situations.
- Prior to testing, there should be a full discussion of the advantages and disadvantages of the procedures, the implications of the possible test results and subsequent management options.

Chromosome analysis

Fetal (placental) cells are examined to look at the number and structure of each chromosome. A full chromosome analysis, called a karyotype, allows the diagnosis of chromosomal abnormalities. A karyotype takes 10 to 14 days and the result will be sent to the referring clinician. Where there are strong indications of a fetal anomaly (for example, very high-risk screening result), FISH analysis (see Appendix 1) may also be performed, which gives a preliminary result in 48 to 72 hours.

Genetic conditions

A couple with a family history of a genetic condition must be referred to a genetics service as soon as possible, preferably prior to pregnancy.

Testing for genetic conditions (for example, cystic fibrosis or thalassaemia) requires knowledge of the causative genetic mutations in the family. This may require extensive, time-consuming tests before a prenatal diagnosis can be offered. Referral once the woman is pregnant may be too late to offer prenatal diagnosis.

See <http://www.hgsa.com.au/PDF/HGSALaboratories.pdf> pages 4 to 8 for a list of genetic tests available.

Genetic testing is usually performed on placental cells collected by *chorionic villus sampling*. Prenatal diagnosis of genetic conditions requires the coordination of the sampling procedure, cytogenetic laboratory and DNA laboratory. The woman should therefore be referred to the genetics service as soon as the pregnancy is confirmed. The time until results are available will depend upon the type of test performed.

Chorionic villus sampling (CVS)

- A sample of chorionic villus (pre-placental tissue) is removed by a fine needle, visualised by ultrasound.
- The tissue is used for chromosome analysis and, in specific situations, may be used for prenatal diagnosis of a genetic condition.
- The procedure can be performed from 11 weeks, routinely between 11 and 13 weeks gestation. It should not be performed prior to 10 weeks gestation due to the risk of limb defects.
- It is an earlier procedure than amniocentesis and has the benefits of an early scan. An early scan may detect some neural tube defects.
- Some women experience cramping and, occasionally, some vaginal bleeding after CVS. These symptoms should only last a day or so. If symptoms persist, the woman should contact her doctor.
- The miscarriage risk is estimated to be 1% above the background risk, in experienced hands.
- **Disadvantages include:**
 - ✦ Early detection of some affected pregnancies that may spontaneously miscarry.
 - ✦ A 1% risk equivocal results (for example, mosaicism).⁷
 - ✦ A 0.1% failure to detect a pregnancy with a chromosome abnormality. This arises when there is an abnormal karyotype in the baby but not the placenta.

Amniocentesis

- A sample of amniotic fluid is removed by a fine needle, visualised by ultrasound.
- The procedure is performed from 15 weeks, routinely to approximately 20 weeks gestation.
- Discomfort is usually minimal, though a very small number of women experience pain as the needle passes through the peritoneum.
- The miscarriage rate is estimated to be 0.5% above the background risk, in experienced hands, which is lower than that for CVS.

⁷ Chromosome mosaicism is the presence of mixture of cells with normal and abnormal karyotype. Where mosaicism is found on CVS, amniocentesis may be necessary to clarify the karyotype of the fetus.

- As amniocentesis is a second trimester test, the pregnancy is more advanced compared to CVS when results are available.
- The fetal cells in the sample are used for chromosome analysis.
- Alpha fetoprotein (AFP) levels may also be measured in the amniotic fluid. If AFP levels are raised, an experienced operator should perform a detailed ultrasound scan for fetal anomalies, examining the neural tube and the abdominal wall in detail. Raised AFP levels are an indication of a possible open neural tube defect or other open birth defect; for example, gastroschisis or exomphalos. Levels will not be raised if a layer of skin covers the neural tube defect. About 5% of neural tube defects will be missed by determination of AFP levels.
- Amniocentesis is not the preferred screening test for neural tube defects. An ultrasound scan at 18 to 20 weeks' gestation is more accurate.

Arranging CVS and amniocentesis

- Refer to a private ultrasound practice or public hospital ultrasound department that performs sufficient procedures per year to have experienced operators.
- The woman's blood group should be given on the request, as rhesus negative women will require an anti-D injection.
- There are no out-of-pocket costs in the public system if there is indication for testing.
- For private patients, there are costs for both the sampling procedure and chromosome analysis. Contact service providers for details.

Managing a pregnancy with an abnormal karyotype

See also Part 1 *Talking with families*.

- It may be helpful to discuss the results with the service provider or clinical geneticist prior to informing the woman.
- Listen and give the woman time to absorb the news and consider her options
- **Referral to a specialist genetic service** is recommended for sex chromosome abnormalities and mosaic results and should be considered for other abnormal karyotypes.
- Not all chromosome abnormalities have a major effect on the baby. Medical texts are often out of date. It is important that the couple receive up-to-date, unbiased information about the potential effects of a chromosome abnormality. Discussion with support groups can be helpful for parents.
- Decisions regarding the pregnancy should only be made once there has been a full discussion of the implications of the test results and the management options. This might include referral to genetic services, discussion with obstetricians and paediatricians, and referral to the relevant support group.

Detecting fetal anomalies

Second trimester ultrasound scan may detect:

- Neural tube defects (anencephaly, spina bifida)
- Cardiac defects
- Gastrointestinal malformations (gastroschisis, exomphalos)
- Limb defects
- Central nervous system defects
- Soft signs which may be associated with underlying chromosomal or genetic conditions.

Limitations

- *Not all malformations can be detected by ultrasound by a second trimester fetal anomaly scan (18 to 20 weeks).* The sensitivity depends on the nature of the malformation, the experience of the operator, and the capacity of the ultrasound equipment.
- Visualisation of the fetus can be affected by factors such as the woman's build and the position and size of the fetus.
- Second trimester ultrasound scan is *not recommended* as a primary screening test for Down syndrome (Gardner & Sutherland 1996).

- The significance of changes (soft signs of chromosome abnormality) detected by ultrasound may be difficult to interpret. Further investigations may be indicated. Advice can be sought from a specialist ultrasonologist, genetics service or an obstetrician in a high-risk pregnancy management unit.

Neural tube defects

- *The best detection method for neural tube defects is an ultrasound scan during the second trimester (18 to 20 weeks).*
- If a previous pregnancy has been affected by a neural tube defect or a first-degree relative with a neural tube defect, an ultrasound scan at both 12 to 13 weeks and 18 weeks is recommended.
- The identification of a neural tube defect by ultrasound depends on the skill of the operator, the equipment, the position and gestation of the fetus, and maternal conditions.
- Periconceptual folate is recommended to decrease the risk of neural tube defects (Public Health Association of Australia 1998) and 0.5 mg daily is recommended for most women. A higher dose (5mg daily) is recommended for women with a previous pregnancy and/or family history of neural tube defects.

Managing a pregnancy with a fetal anomaly

Referral of public patients to a high-risk clinic, perinatal management unit or fetal diagnostic unit *is strongly recommended* for a management plan and coordination of ongoing care.

Women and families benefit from detailed discussion with support groups and clinicians that have experience in the management of babies with disability and birth defects. These services are located in the public and private sector.

A management plan may include providing the couple with:

- The opportunity for consultation with appropriate specialists.
- Further diagnostic procedures.
- Further ultrasound scans with appropriate expertise present (for example, cardiologist present if cardiac defect is suspected).
- Genetic counselling.
- Ongoing support if the pregnancy continues to term.
- Specific plans for delivery, postnatal care and support.
- Contact with the relevant support groups.

Counselling regarding termination of pregnancy

- It is common for couples to be deeply shocked after receiving bad news. They often immediately request a termination. It is important to allow some time for the couple to come to terms with the bad news and consider their decision. This may include discussions with relevant health professionals and support groups.
- If termination of pregnancy is an option, the couple should be encouraged to make their decision based on their personal values and accurate, up-to-date and unbiased information.
- When couples have a choice of the method of termination, all options and the associated risks and advantages should be discussed, preferably with an obstetrician or prenatal genetic service.
- Grief after a termination is a normal reaction to the loss of a wanted pregnancy and can be complicated by feelings of guilt⁸ and anxiety for future pregnancies.
- Ongoing support for the couple is important, regardless of the couple's decision. Couples may turn to their GP for support, or benefit from consultation with a genetic counsellor with experience in prenatal diagnosis. Support after termination is available through Support After a Fetal Diagnosis of Abnormality.

⁸ Guilt can be expressed through feelings of contributing to the condition (What did I do to cause this?), feelings of punishment (Have I been bad? Am I being punished?) and shame for rejection of the pregnancy. See also Part 1 Talking with families.

5.3 Frequently asked questions

What if the woman presents late?

A woman who is not at increased or high risk of having a baby with Down syndrome and first presents after 20 weeks gestation is limited to an ultrasound scan for fetal anomalies, which should be immediately arranged.

For a woman over the age of 37 years at EDD, an amniocentesis with FISH (see Appendix 1) could be considered up to the end of the 20th week. A FISH analysis could be performed to give a preliminary result within 48 hours of the amniocentesis, with the final karyotype result taking up to two weeks. The pregnancy is then fairly advanced and termination of pregnancy may not be available as an option.

What do I say to a woman who is anxious while waiting for her results?

From the time the test is arranged, it is important that the woman be given realistic expectations, including the maximum period to wait for test results (for example, 14 days if test is cited as taking 10 to 14 days). She should be informed that the time taken to receive results does not indicate if they will be normal or not. Talking about the most likely outcomes can be helpful, but avoid false reassurance. Often listening to the woman's anxieties and using counselling skills such as active listening, normalising and acknowledging the distress and uncertainty are useful. Make information available if the woman requests more.

What do I do if a woman says there is 'something abnormal' on a test result but I haven't received them yet?

Contact the genetic counsellor or geneticist at the hospital or ring Genetic Health Services Victoria. They will be able to direct you or inform you of the situation.

What do I say to a woman whose Down syndrome risk has increased, but is still below the cut-off for a diagnostic procedure?

Explore the meaning of this result for the woman. While her risk is greater than other women her age, her risk of having a baby with Down syndrome is still low. Her chance of having a baby with Down syndrome is less than the risk of miscarrying from the procedure.

5.4 Correcting misunderstandings

'I'm young, so I don't need to have any tests for Down syndrome'

Many babies with Down syndrome are conceived by women less than 37 years of age. Screening tests should be offered to young women. These screening tests identify many but not all women who may be at increased risk and eligible for diagnostic testing.

'The tests were all OK, so my baby is normal'

Tests during pregnancy can detect only certain conditions. No test, or combination of tests, will detect all birth defects or medical conditions.

'My blood test was normal, so my baby doesn't have Down syndrome'

Blood tests cannot detect all pregnancies with Down syndrome. A woman with a "normal" (low risk) blood test result does have a chance of having a baby with Down syndrome but this risk is not high enough for diagnostic testing to be indicated.

'The blood test says there's something wrong with my baby'

Blood tests during pregnancy do not detect birth defects; they indicate which pregnancies have an increased risk of certain genetic conditions and birth defects. Most fetuses with 'abnormal' (increased risk) test results do not have Down syndrome. This is an indication for referral for diagnostic procedures.

'The blood test says there is something wrong so I need a diagnostic test'

If the blood test was second trimester maternal serum screening, increased risk results are due to inaccurate dates (if LNMP only given) in 30% of cases. Check dates by ultrasound.

An increased risk result on a screening test is an indication for diagnostic testing; however, a small number of women choose not to have diagnostic testing.

'If I have another screening test, I might get a better result'

Screening tests are most accurate when done at the correct time in the pregnancy. Retesting is only performed if dates are inaccurate.

'I am over 37 years, so I need to have a CVS or amniocentesis'

Women over 37 years at EDD may choose to have a diagnostic test, may prefer the option of screening tests, or have no tests at all. Women in this age group should be aware that they are more likely to have an increased risk result from a screening test as age is part of the risk calculation. They will then need to consider diagnostic testing.

'It's not worth having the test because I wouldn't terminate the pregnancy'

Some people feel it is beneficial to know if the fetus has a birth defect to prepare for the birth and future. Others prefer to wait until delivery. All women should have the opportunity to consider testing.

Appendix 1: FISH

What is FISH?

- FISH stands for fluorescent *in situ* hybridisation and is used to visualise specific chromosomes.
- FISH can be applied to samples collected by CVS or amniocentesis. It provides an *interim result* and a complete chromosome analysis should still be completed. FISH relies on DNA fragments ('probes') with fluorescent molecules attached. These probes are specific for particular chromosomes. For example, chromosome 21 probe will bind to a specific location on chromosome 21. The fluorescent molecules allow the chromosome to be visualised using a fluorescent microscope, while other chromosomes cannot be seen. The number of chromosomes bound by the probe can then be counted. In this way, a cell with an extra copy of, for instance, chromosome 21 (trisomy 21) can be recognised.

What can FISH detect?

- FISH will detect some chromosome abnormalities. The number of probes used will determine the type of chromosome abnormalities detected.
- 'Three-probe FISH' will detect *trisomy 21* (Down syndrome) and *sex chromosome abnormalities*.
- 'Five-probe FISH' will detect *trisomy 21* (Down syndrome), *trisomy 18* (Appendix 2), *trisomy 13* (Appendix 3) and *sex chromosome abnormalities*.

What is the advantage of FISH?

- FISH gives a quick preliminary result for the presence of trisomy 21 and certain other chromosome trisomies (see above).

What are the limitations of FISH?

- FISH can give false positive results; therefore, an abnormal result needs to be interpreted cautiously if other indications of trisomy are not present.
- *FISH does not replace a complete chromosome analysis.*
- FISH results may be inconclusive if both normal and abnormal cells are present (mosaicism).

Who may benefit from FISH?

- **Indications for FISH include:**
 - ✦ Fetal anomaly detected on routine second trimester ultrasound scan.
 - ✦ Very high-risk result on a screening test. This would be a risk of one in 20 of Down syndrome or greater.
 - ✦ Late gestation.
- A private patient may elect to have a FISH test.

How is FISH arranged?

- FISH is requested by the obstetrician or the ultrasonologist.

How much does FISH cost?

- There is no Medicare rebate for FISH and private patients will be charged.
- The charge depends on the laboratory doing the testing and the number of probes used.
- Costs for three-probe FISH may be less than for five-probe FISH.
- Contact the pathology or ultrasound service for details of costs.

How long do results take?

- Results are available in 24 to 48 hours.
- The referring doctor, antenatal clinic or genetics service will be informed of the results.

Appendix 2: Trisomy 18

Also known as Edwards syndrome.

What is trisomy 18?

Trisomy 18 is the presence of an extra copy of chromosome 18 in all cells of the body.

Clinical features

Trisomy 18 is associated with severe developmental delay, intellectual disability and multiple malformations including:

- Cardiac defects
- Urinary tract and renal malformations
- Joint contractures
- Low birth weight
- Hearing loss
- Rockerbottom feet.

What is the outlook?

Of fetuses diagnosed with trisomy 18 at amniocentesis, approximately 70% spontaneously abort before birth (Gardner & Sutherland 1996).

Those that are liveborn have severely shortened lifespans. Of those babies who are liveborn, 30% die in the first month of life and 90% die by 12 months.

The prognosis for babies with partial or mosaic trisomy 18 can be less severe. For further information, contact a genetics service.

Risks of trisomy 18

- The risk of trisomy 18 increases with maternal age.
- The risk of recurrence is low. For women of advanced maternal age, the risk remains the age-related risk of a trisomy. For younger women, there may be a slight increase in the risk of a trisomy.

Appendix 3: Trisomy 13

Also known as Patau syndrome.

What is trisomy 13?

Trisomy 13 is the presence of an extra copy of chromosome 13 in all cells of the body.

Clinical features

- Craniofacial malformations such as cleft lip and/or palate
- Ocular malformations (microphthalmia)
- Neurological malformations (for example, holoprosencephaly, neural tube defects)
- Limb anomalies (postaxial polydactyly)
- Cardiovascular anomalies
- Ventral wall defects (omphalocele)
- Diaphragmatic defects
- Urinary tract and renal anomalies (hydronephrosis, cystic kidneys).

In addition, they are severely intellectually disabled.

What is the outlook?

Of fetuses diagnosed with trisomy 13 at amniocentesis, approximately 45% spontaneously abort before birth (Gardner & Sutherland 1996).

The average length of survival of an infant with trisomy 13 is approximately four days, usually due to the presence of cardiac defects.

The prognosis for babies with partial or mosaic trisomy 13 can be less severe. For further information, contact a genetics service.

Risks of Trisomy 13

- The risk of trisomy 13 increases with maternal age.
- The risk of recurrence is low. For women of advanced maternal age, the risk remains the age-related risk of trisomy. For younger women, there may be a slight increase in the risk of a trisomy.

Appendix 4: Pre-implantation genetic diagnosis

What is pre-implantation genetic diagnosis (PGD)?

PGD is the genetic testing of embryos prior to implantation in the womb and relies on IVF or ICSI technology to generate embryos in vitro. Embryos are tested at day 3 (six to 10 cells) after fertilisation. One or two cells are removed and used for testing.

What tests can be performed?

- Identification of sex.
- FISH analysis (see Appendix 1) to identify chromosome trisomies.
- Selected single gene disorders.

Prerequisites for testing for single gene disorders

- The gene changes causing the condition must be known.
- It must be established that testing for the gene alteration is accurate on a single cell.

Advantages of PGD

- If a woman and her partner are trying to avoid a pregnancy affected with a certain genetic condition, the risk of this can be minimised without termination of the pregnancy.
- Referral for specialised counselling is required. This provides a forum to discuss issues in detail with an experienced counsellor who has been trained in this area.

Limitations of PGD

- There is no guarantee of achieving a pregnancy.
- Accuracy is high, but not 100%.
- The woman must undergo IVF procedures.
- The procedures and testing are expensive.
- It is time consuming and requires meticulous lab work.
- There is a risk of multiple pregnancy as more than one embryo may be implanted.

Counselling issues

- Risks and success rates of PGD must be made clear in relation to other methods of avoiding genetic risk.
- There can be personal moral dilemmas regarding the use of embryos not implanted.
- Grief and loss.
- Attitude to prenatal diagnosis.

5.5 Patient and further information

Websites

Better Health Channel:

<http://www.betterhealth.vic.gov.au>

Relevant topics:

- Maternal serum screening
- Amniocentesis
- Ultrasound – scan
- Down syndrome is a common genetic condition
- Birth defects involving chromosomes – trisomy disorders

RACGP 2001, *Guidelines for preventative activities in general practice* at:

<http://www.racgp.org.au/publications>

DHS 2001, *The three centres consensus guidelines on antenatal care* at:

<http://www.dhs.vic.gov.au/ahs/quality/effect.htm>
(under Maternity Care)

Royal Women's Hospital, Sunshine Hospital, Mercy Hospital for Women 2002, *Guidelines for shared maternity affiliates* at:

<http://www.mercyhealth.net/>
<http://www.rch.unimelb.edu.au>
<http://www.wh.org.au>

Royal Australian and New Zealand College of Obstetricians and Gynaecologists:

<http://www.ranzcog.edu.au>

DHS, *Guidelines for the control of infectious diseases* at:

http://www.dhs.vic.gov.au/phd/hprot/inf_dis/bluebook

Down Syndrome Association Victoria:

<http://www.dsav.asn.au>

Human Genetics Society of Australasia:

<http://www.hgsa.com.au>

Further reading

de Crespigny, L, Espie, M & Holmes, S 1998, *Prenatal testing: making choices in pregnancy*, Penguin Books, Melbourne.

Riley, M & Halliday, J 2000, *Birth defects in Victoria (1983–1998)*, Perinatal Data Collection Unit, Department of Human Services Victoria.

Sheffield, L 2002, 'Prenatal screening and diagnosis of genetic disorders', *Current Therapeutics*, 43:12–18.

Webley, C & Halliday, J 2002, *Report on prenatal diagnostic testing in Victoria in 2001*, Perinatal Data Collection Unit, Department of Human Services Victoria.

5.6 Where to refer

Credentialed ultrasound practitioners

Please see Royal Australian and New Zealand College of Obstetrics and Gynaecology website for list: <http://www.nuchaltrans.edu.au/>

Maternal serum screening information and counselling

Serum Screening Program
Genetic Health Services Victoria
10th Floor, Royal Children's Hospital
Flemington Road, Parkville 3052
Call: (03) 83421 6303
Fax: (03) 8341 6390

Genetic Counselling Services

Royal Women's Hospital
Genetic Health Services Victoria
Grattan Street, Carlton 3053
Call: (03) 9344 2121
Fax: (03) 9344 2066

Monash Medical Centre
Clinical Genetics/Genetic Health Services Victoria
Clayton Road, Clayton 3168
Call: (03) 9594 2026
Fax: (03) 9594 2022

Royal Children's Hospital
Genetic Health Services Victoria
Flemington Road, Parkville 3052
Call: (03) 8341 6270
Fax: (03) 8341 6390

Mercy Hospital for Women
Genetics Department
Clarendon Street, East Melbourne 3002
Call: (03) 9270 2394
Fax: (03) 9270 2498

Rural and regional services (Ballarat, Bendigo, Frankston, Geelong, Mildura, Sale, Shepparton, Traralgon, Warragul, Warrnambool)

Genetic Health Services Victoria
Call: (03) 8341 6201
Fax: (03) 8341 6390

Albury/ Wodonga Genetics Clinic
Genetic Health Services Victoria
78 Vermont Street, Wodonga 3690
Call/Fax: (02) 6056 0451

Fetal management units (public multidisciplinary services)

Fetal Management Unit
Royal Women's Hospital
132 Grattan Street, Carlton 3053
Call: (03) 9344 2709
Fax: (03) 9347 8790

Fetal Diagnostic Unit
Monash Medical Centre
Clayton Road, Clayton 3168
Call: (03) 9594 2343
Fax: (03) 9594 6226

Please note that some private ultrasound services provide multidisciplinary services upon detection of a fetal anomaly.

Support groups

Support After Fetal Diagnosis of an Anomaly (SAFDA)
A peer support group for parents who have had a termination of pregnancy after diagnosis of a fetal anomaly
No postal address
Call: (03) 9344 2121
Fax (03) 9344 2066

Support Organisation For Trisomies (SOFT)
Karin Schuler
198 Oak Road, Kirrawee NSW 2232
Call: (02) 9521 6039
Fax: (02) 9542 5305

Down Syndrome Association
495 High Street, Northcote 3070
Call: (03) 9486 2377
Fax: (03) 9486 2435
Email: dsavic@netspace.net.au
Website: <http://www.dsav.asn.au>

Information about other support groups is available from Genetic Support Network Victoria on (03) 8341 6315 or visit the website: <http://www.gsnv.org.au>

5.7 References

Crossley, JA, Aitken, DA, Cameron, AD, McBride, E & Connor, JM 2002, 'Combined ultrasound and biochemical screening for Down's syndrome in the first trimester: a Scottish multicentre study', *B J Ob Gyn*, 109(6):667–76.

Cuckle, HS & van Lith, JM 1999, 'Appropriate biochemical parameters in first-trimester screening for Down syndrome', *Prenatal Diagnosis*, 19:505–12.

Gardner, RJ & Sutherland GR 1996, *Chromosome abnormalities and genetic counselling*, Oxford University Press.

Halliday, J, Warren, R, MacDonald, G, Liamputtong, Rice, P, Bell, R & Watson, L 2001, 'Prenatal diagnosis for women 37 years and over: to have or not to have', *Prenat Diag*, 21:842–47.

RANZCOG & HGSA 2001, *Antenatal screening for Down syndrome and other fetal aneuploidy*, College Statement C-Obs 4: http://www.hgsa.com.au/policy/Antenatal_Screening.html

Sheffield, L 2002, 'Prenatal screening and diagnosis of genetic disorders', *Current Therapeutics*, 43:12–18.

Webley, C & Halliday, J 2002, Report on prenatal diagnostic testing in Victoria in 2001, Perinatal Data Collection Unit, Department of Human Services Victoria.

Public Health Association of Australia 1998, *Periconceptual folate and the prevention of neural tube defects*, policy statement: http://www.phaa.net.au/policy/frame_policyinitiatives.html

