THE JOURNAL OF
Obstetrics and Gynaecology Research

doi:10.1111/jog.13070 J. Obstet. Gynaecol. Res. Vol. 42, No. 10: 1229–1235, October 2016

Chorionic villous sampling through transvaginal ultrasound approach: A retrospective analysis of 1138 cases

Mohan S. Kamath[1], Sujata Pradhan[1], Eunice Sindhuvi Edison[2], Shaji Ramachandran Velayudhan[2], Belavendra Antonisamy[3], Muthukumar Karthikeyan[1], Ann M. Mangalaraj[1], Aleyamma Kunjummen[1] and Korula George[4]

[1]Reproductive Medicine Unit, Departments of [2]Haematology, and [3]Biostatistics, Christian Medical College, Vellore and [4]Reproductive Medicine Unit, Bangalore Baptist Hospital, Bangalore, India

Abstract

Aim: The aim of this study was to evaluate the effectiveness and safety of a transvaginal approach for chorionic villous sampling (CVS).

Methods: We carried out a retrospective data analysis of all the transvaginal CVS procedures performed for the purpose of prenatal diagnosis in a university-level referral center between January 2000 and December 2014. Women underwent the prenatal testing between 10 and 17 weeks of gestation mainly for hematological disorders involving single gene defects. The main outcomes were successful sampling rate, maternal contamination rate, post-procedure complications rates, and immediate fetal loss rate (<14 days post-procedure).

Results: A total of 1138 transvaginal CVS were performed during the study period and were available for analysis. The sampling success rate after the first attempt was 98.5% (1121/1138) and the overall success rate was 99.6% (1133/1138). The maternal contamination rate was 0.4% (5/1138). While two patients had vaginal bleeding (0.2%), fresh retroplacental collection was noted in four patients (0.4%) post-procedure. None of the patients developed ascending uterine infection following CVS. The immediate fetal loss rate was 0.2% (2/1138).

Conclusion: Transvaginal approach is associated with high sampling success, along with low rates of maternal contamination and post-procedure complications; hence, it can be offered as an effective alternative method of CVS.

Key words: chorionic villous sampling, molecular genetics, CVS.

Introduction

Worldwide, chorionic villous sampling (CVS) and amniocentesis are the most commonly performed prenatal diagnostic procedures for a definitive diagnosis of fetal genetic and chromosomal disorders. Conventionally, CVS is done either by a transabdominal or a transcervical route.[1] Even though the transabdominal CVS route is considered safer than the transcervical approach, it can be technically difficult in obese women and in those who have a posterior placenta.[2,3] The transcervical route has been associated with multiple insertions, risk of vaginal bleeding and ascending vaginal infection.[4] Ultimately, the selection of approach for prenatal diagnostic tests is based on the clinician's training and preferences.[5]

The fetal loss rates following transabdominal CVS have been found to be similar to that of second-trimester amniocentesis.[2] The main concern with late amniocentesis is the delay in obtaining the final diagnosis, which can be distressing for the anxious couple.[1] The increased morbidity associated with pregnancy termination for an affected fetus at a more advanced gestation is of particular concern for the clinician and the patient. The widespread usage of first-trimester fetal aneuploidies screening increases the number of women in need of

Received: January 30 2016.
Accepted: May 2 2016.
Correspondence: Dr, Professor and Head, Korula George, Reproductive Medicine Unit, Bangalore Baptist Hospital, Hebbal, Bangalore 560024, India.
Email: gkorula@gmail.com

invasive prenatal testing for positive screening results. In these circumstances, a first-trimester CVS would be a more preferred option than second-trimester amniocentesis.[6] Hence, there is a felt need for a safer first-trimester prenatal diagnostic test as an alternative to late amniocentesis.

There have been a few studies exploring the feasibility and safety of a transvaginal approach for CVS.[3,7,8] We published a small series of 38 women who underwent transvaginal CVS for prenatal diagnosis of hematological disorders and found the procedure safe and technically less challenging than transabdominal CVS.[9]

We planned to do a retrospective analysis of the women who underwent transvaginal CVS in our unit to further validate this alternative CVS technique.

Methods

We conducted a retrospective data analysis of all the transvaginal CVS procedures done for the purpose of prenatal diagnosis in our unit between January 2000 and December 2014. Institutional review board approval was obtained prior to the study. Women who were referred to our unit for prenatal diagnosis of monogenic hematological diseases, such as β-thalassemia, hemophilia, sickle cell disease, Glanzmann thrombasthenia, leucocyte adhesion defect, and Wiskott–Aldrich syndrome, were included in the study. In some cases, karyotype analysis for chromosomal abnormalities was also done concurrently. In a few cases, only karyotype analysis was done for ruling out chromosomal abnormalities.

We excluded women with multiple pregnancy, those with history of vaginal bleeding in the week prior to the planned procedure, and those with a gestation period of >17 weeks.

A transvaginal ultrasound was done to: (i) confirm a viable intrauterine pregnancy; (ii) estimate the period of gestation; (iii) localize the placenta (anterior, posterior, or fundal); and (iii) identify any existing subchorionic collection.

We delayed the CVS until 10 completed weeks of gestation as there have been concerns regarding possible association between early CVS (<9 weeks) and limb reduction defects.[10] Written informed consent was obtained prior to the procedure.

For the procedure, a 17-gauge sterile IVF ovum pick-up needle (Cook Australia) was directed, using a needle guide that was mounted on a 5-Mz transvaginal ultrasound probe, towards the predetermined placental site. The needle was carefully moved back and forth through the placenta while simultaneously applying suction with the syringe. The negative pressure was continuously maintained while the needle was being withdrawn (Fig. 1).

The flushed chorionic tissue was collected in a petri dish (saline with 10 Mm ethylenediaminetetraacetic acid) and separated from maternal tissue under a stereomicroscope. When a concurrent karyotype analysis was also being planned, an additional petri dish with only saline was used. The chorionic tissue was weighed and transported to the molecular hematology laboratory. A repeat ultrasound was performed after 24 h to confirm fetal cardiac activity. The final mutation analysis was provided to the patient within 1 week. The follow-up visit was planned within a fortnight as per unit protocol. Patients were encouraged to report any symptoms, such as bleeding per vaginum, pain in the abdomen, watery discharge, or fever, during the follow-up visit. In case of emergence of any symptoms during the follow-up period of up to 2 weeks, the patient was advised to report to the unit for further management. Subsequently, the patients with fetuses without any abnormal mutation analysis and continuing pregnancy returned to their original obstetric unit for antenatal care.

DNA was extracted from chorionic villus sample using the Puregene Gentra kit (Qiagen) method. The reverse dot blot method was used to screen for common beta thalassemia mutations and the rare mutations were screened by DNA sequencing analysis.[11] In hemophilia A, the common inversions were screened by inverse polymerase chain reaction (PCR).[12] Other mutations were screened by conformation sensitive gel electrophoresis

Figure 1 Retroverted uterus with posterior placenta and a needle being directed towards placenta using the needle guide mounted on a transvaginal ultrasound probe.

(CSGE) and DNA sequencing. In all other diseases, the genetic defect was identified using DNA sequencing. Gross deletions were analyzed by GAP PCR and gene dosage analysis. Maternal contamination of the fetal tissue was analyzed by amplifying a panel of six VNTR markers using fluorescent-labeled primers followed by capillary electrophoresis.[13]

We analyzed the study outcomes using the following variables:

1. Successful attempt (ability to retrieve adequate amount of chorionic tissue required for analysis without maternal contamination). Amount of tissue retrieved was grouped as <4 mg, 4–20 mg, >20 mg.
2. Number of needle insertions to retrieve minimum required amount of chorionic tissue (1/2/>2 insertions in the same attempt).
3. Need for second attempt due to unsuccessful first attempt.
4. Maternal tissue contamination that may adversely affect fetal genetic diagnosis.
5. Vaginal bleeding other than minimal spotting that was commonly observed following the procedure.
6. Appearance of fresh retroplacental collection as assessed by ultrasound post-CVS.
7. Miscarriage (immediate ≤14 days and remote >14 days).
8. Evidence of intrauterine infection or chorioamnionitis following the procedure (fever, foul smelling discharge per vaginum).

The data were entered using Epidata and further analysis was done using STATA version 13. All of the categorical variables were reported as frequencies and percentages. Continuous variables were summarized as mean ± standard deviation (SD) or median with interquartile range (IQR). The χ^2-test was used to examine the association between categorical exposure variables and successful tissue retrieval.

Results

A total of 1138 transvaginal CVS were performed during the study period between January 2000 and December 2014 and were available for analysis.

The mean age of patients undergoing CVS was 28.4 ± 4.7 years (Table 1).

The CVS was successful (adequate chorionic tissue without maternal contamination) in the first attempt in 1121 out of 1138 (98.5%) cases. A total of 17 cases (1.5%) required a second attempt due to either inadequate tissue retrieval (n = 12) or maternal contamination (n = 5). Out of the 12 unsuccessful first attempts (due to inadequate tissue), 11 had successful second attempts (eight transvaginal; three transabdominal). One remaining patient underwent a repeat attempt that yielded sample with maternal tissue contamination. Among the five cases with maternal contamination, four had successful transvaginal retrieval in the second attempt and one had successful transabdominal CVS. The overall success rate (combining first and second attempts) of the transvaginal CVS was 99.6% (1133/1138) (Table 1). The maternal contamination rate was 0.4% (5/1138).

The data regarding post-procedure complications were available for 1125 procedures. Two patients had

Table 1 Maternal characteristics and sample weight and CVS outcomes (n = 1138)

Maternal characteristics related to CVS procedure	*n* (%)
Age (years)†	28.4 (± 4.7)
Period of gestation (weeks)†	11.3 (± 1.7)
Tissue weight (mg)‡	26 (15–44)
Number of successful tissue retrievals after first attempt	1121 (98.5%)
Number of successful tissue retrievals after first and second attempts (overall success rate)	1133 (99.6%)
Maternal tissue contamination	5 (0.4%)
Number of women with fetal loss immediately post-procedure (<14 days)	2 (0.2%)
Number of women with vaginal bleeding post-procedure (n = 1125)	2 (0.2%)
Number of women with leaking per vaginum immediately post-procedure (n = 1125)	1 (0.1%)
Number of women with fresh retroplacental collection immediately post-procedure (n = 1125)	4 (0.4%)
Weight of tissue retrieved (mg) (n = 1123)	
<4	32 (2.9%)
4–20	417 (37.1%)
>20	674 (60.0%)

Values are reported as n (%) for categorical variables. †Mean (± SD) for continuous variables. ‡Median and interquartile range.

frank vaginal bleeding after the procedure (0.2%). None of the women had evidence of intrauterine infection following transvaginal CVS. While 31 women had pre-existing retroplacental collection before the planned procedure, four women (0.4%) had evidence of fresh retroplacental collection. One patient developed leaking per vaginum post-procedure (Table 1).

Two patients (2/1138 = 0.2%) had loss of fetal cardiac activity detected during post-procedure ultrasound. One of the women, who underwent CVS at 10 weeks of gestation, developed leaking per vaginum at 17 weeks, which was managed conservatively. The patient subsequently had preterm delivery at 28 weeks and the neonate had respiratory morbidity.

Successful retrieval rates for women in the age groups of ≤25, 26–29, 30–34, and ≥35 years were 98.4%, 99.5%, 98.1%, and 96.9%, respectively, with no significant difference across the age groups ($P = 0.17$) (Table 2).

Information regarding number of needle insertions during a single attempt was available for 1134 procedures. The successful tissue retrieval rate for one, two, and three needle insertions during the same attempt was 99.4% (821/826), 96.4% (270/280), and 96.4% (27/28), respectively. The chances of successful retrieval were significantly lower if more than one insertion was attempted ($P < 0.001$) (Table 2).

Data were available regarding the period of gestation for 1127 procedures. The mean gestational age at which the procedure was planned was 11.3 ± 1.7 weeks. The successful retrieval rate in women with gestational stages of 10–12, 13–14, and ≥15 weeks was 98.6% (921/934), 97.7% (130/133), and 100% (60/60), respectively, with success being similar across all of the groups ($P = 0.47$) (Table 2).

The data for weight of chorionic tissue retrieved were available for 1123 procedures. For 15 procedures, the weight of tissue was not available, but the mutation analysis report was available, thereby confirming the adequacy of tissue for analysis and ruling out maternal contamination. The median (IQR) weight of sample tissue was 26 (15–44) mg. Less than 4 mg tissue was retrieved for 32 cases (2.9%), 4–20 mg for 417 cases (37.1%), and >20 mg was retrieved for 674 cases (60.0%) (Table 1). Among the 32 patients with <4 mg, only 12 required a repeat attempt for inadequate sample.

The placental location data were available for 1072 cases. The successful tissue rate was 99.4% (535/538), 97.3% (466/479), and 100% (55/55) for the anterior, posterior, and fundal positions, respectively. The chances of retrieval were significantly lower when the location was posterior compared to anterior or fundal ($P = 0.02$) (Table 2).

A total of 906 CVS were carried out for prenatal diagnosis of single-gene disorders and for 210 cases, both DNA and cytogenetic analysis were conducted. Only 22 patients underwent CVS for cytogenetic analysis alone.

Table 2 Association between maternal age, number of needle insertions, period of gestation, placental location and successful tissue retrieval

Variables	Successful tissue retrieval *n* (%)			*P*-value†
	Yes	No	Total	
Age (years)				
≤25	316 (98.4)	5 (1.6)	321 (28.2)	
26–29	375 (99.5)	2 (0.5)	377 (33.1)	0.17
30–34	304 (98.1)	6 (1.9)	310 (27.2)	
≥35	126 (96.9)	4 (3.1)	130 (11.4)	
Number of needle insertions during one session				
1	821 (99.4)	5 (0.6)	826 (72.8)	<0.001
2	270 (96.4)	10 (3.6)	280 (24.7)	
3	27 (96.4)	1 (3.6)	28 (2.5)	
Period of gestation at which CVS was performed (weeks)				
10–12	921 (98.6)	13 (1.4)	934 (82.9)	0.47
13–14	130 (97.7)	3 (2.3)	133 (11.8)	
15+	60 (100.0)	0 (0.0)	60 (5.3)	
Placental location				
Anterior	535 (99.4)	3 (0.6)	538 (50.2)	
Posterior	466 (97.3)	13 (2.7)	479 (44.7)	0.02
Fundal	55 (100.0)	0 (0.0)	55 (5.1)	

†*P*-value is obtained by comparing successful tissue retrieval (yes vs no) using the χ^2-test. CVS, chorionic villous sampling.

Discussion

The retrospective analysis of our data revealed a successful sampling rate of 98.5% for transvaginal CVS in the first attempt. The overall successful sampling rate of the transvaginal technique was 99.6% after the first and second attempts.

Our overall successful sampling rate is comparable to the results obtained by conventional transabdominal and transcervical approaches for CVS in earlier studies.[14,15] In a randomized trial comparing transabdominal and transcervical approaches for CVS, the overall success rate was 99.7% and 99.4%, respectively.[14] In a larger randomized trial comparing transcervical and transabdominal route for CVS, the sampling success following first attempt was 98% and 99%, respectively, with an overall success rate of >99%.[15]

Maternal cell contamination in the retrieved sample can lead to error in the diagnosis; hence, it is an important issue from the laboratorial and clinical perspectives. In our study, the maternal contamination rate was 0.4%, which is quite low. In a study evaluating the maternal cell contamination rate following prenatal testing for thalassemia using the quantitative fluorescence PCR, the investigators found a contamination rate of 3.1% (3/98) in the chorionic villous samples.[16] In the large randomized trial by Jackson *et al.*, the maternal tissue contamination rates were 2% and 1% following transcervical and transabdominal CVS, respectively.[15]

In the majority of the women the tissue retrieved was considered adequate for DNA analysis. In a small fraction of our patients, the tissue retrieved weighed <4 mg (2.9%). However, even in those cases where the tissue retrieved was <4 mg, DNA analysis was possible in the majority of cases (20/32) and did not require a second attempt. Unlike many earlier studies where the main indication for prenatal diagnosis was chromosomal abnormalities, the main indications for CVS in our study were single gene disorders.[14,15] The sample tissue required for DNA analysis is less compared to karyotype analysis. Hence, the high sampling success rate in our study may be partly attributed to higher proportion of single gene disorder indications. Further data are needed to validate the technique for prenatal karyotype analysis. Among the factors affecting the success of transvaginal CVS, we found no association with age of the patient or period of gestation at which CVS was performed. However, we found that the posterior placental position was associated with a significantly lower sampling rate ($P = 0.02$) and the sampling success rate, even with a posterior placenta, was high (97.3%). Similarly, significantly lower successful retrieval was obtained when the number of needle insertions increased beyond one ($P < 0.001$). A greater number of insertions is also associated with increased risk of post-procedure complications.[17]

The immediate fetal loss rate was 0.2% and this could be considered as the most likely procedure-related loss, although spontaneous pregnancy loss cannot be completely ruled out. The follow-up period was up to 2 weeks post-procedure, hence the true miscarriage rate (fetal demise, spontaneous miscarriage, or still birth before period of viability) was not available. Earlier trials have revealed fetal loss rates of 1.65–2% and 2.16–3% following transabdominal and transcervical CVS, respectively, which reflects the procedure-related losses and spontaneous losses up to the period of viability.[15,18] The lack of data regarding the true fetal loss rate and obstetric outcome is one of the limitations of our study.

Post-procedure complications, such as vaginal bleeding (0.2%), appearance of fresh retroplacental collection (0.4%), and leaking per vaginum (0.1%), were low. The incidence of post-procedure vaginal bleeding following transcervical CVS has been found to be around 10%.[1] We did not have any cases of post-procedure uterine infection. In a study by Silvermann *et al.*, post-procedure transient bacteremia incidence was found to be 4.1% (2/49) following transcervical CVS, whereas no case had bacteremia after transabdominal procedures ($n = 65$).[19] The reported risk of uterine infection following transcervical CVS was 0.13%.[4] Even though there are similarities in the transvaginal and transcervical approaches (instruments traversing the endogenous microbe-filled vaginal flora), we did not find any case of ascending uterine infection, which has been associated, though rarely, with transcervical CVS.[20]

The conventional transabdominal approach for CVS is considered challenging in obese women and those with posterior placental location in a retroverted uterus. The transvaginal approach was explored by Sidransky *et al.* for cases where chorionic tissue could not be obtained by conventional methods.[21] Shulman *et al.* suggested use of transabdominal ultrasound for transvaginal CVS instead of endovaginal ultrasound guidance for better outcomes.[8] In our earlier case series, we found the technique effective and safe with no major complications being reported.[9] Transcervical CVS involves insertion of the cannula through the cervix, which is technically difficult and has a longer learning curve.[4] We found the learning curve shorter for the transvaginal approach. Our familiarity with assisted reproductive technology-related procedures, such as transvaginal oocyte retrieval

and early pregnancy ultrasound, helped us gain the necessary skills to carry out the procedure effectively, as has also been suggested by an earlier study.[7] Presence of fibroids or adenomyosis did not interfere with tissue retrieval. In most cases, we could find a plane avoiding the fibroids while traversing the myometrium. We found that the transvaginal approach combines the advantages of the transcervical approach (less discomfort, easier access in obese patients and in those with posterior placenta) and the transabdominal method (technically less challenging and shorter learning curve).[4,22]

The limitation of our study was its retrospective design. The shorter post-procedure follow-up, which may have led to underestimation of procedural complications, such as fetal loss rate, hence needs cautious interpretation. The lack of objective assessment of pain relief was also a drawback.

Our study is one of the largest studies to date evaluating the effectiveness and safety of the transvaginal approach for CVS. We obtained a high sampling success rate and very low incidence of maternal tissue contamination. The post-procedure complication rates and immediate fetal loss rates were also low. Importantly, the transvaginal approach has the distinct advantages of both the transcervical and transabdominal methods. However, further prospective studies with long-term follow-up are needed to determine the true miscarriage rate and obstetric outcome in order to further validate this alternative technique.

Acknowledgments

We would like to acknowledge the help of all technical staff working in the areas of hemoglobinopathies and bleeding disorders in the Molecular Lab, Department of Haematology and Reproductive Medicine Unit, Christian Medical College, Vellore.

Disclosure

None declared.

References

1. Alfirevic Z, Mujezinovic F, Sundberg K. Amniocentesis and chorionic villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev* 2003; **3**: CD003252.
2. Smidt-Jensen S, Permin M, Philip J *et al.* Randomised comparison of amniocentesis and transabdominal and transcervical chorionic villus sampling. *Lancet* 1992; **340**: 1237–1244.
3. Sidransky E, Black SH, Soenksen DM, Jones SL, Dorfmann AD, Schulman JD. Transvaginal chorionic villus sampling. *Prenat Diagn* 1992; **12**: 229–234.
4. Brambati B, Lanzani A, Tului L. Transabdominal and transcervical chorionic villus sampling: Efficiency and risk evaluation of 2,411 cases. *Am J Med Genet* 1990; **35**: 160–164.
5. Alfirevic Z, von Dadelszen P. Instruments for chorionic villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev* 2003; **1**: CD000114.
6. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: A systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015; **45**: 16–26.
7. Schulman JD, Black SH, Soenksen D, Jones SL, Dorfmann AD. Transvaginal chorionic sampling during the first trimester of pregnancy. *N Engl J Med* 1988; **319**: 1612.
8. Shulman LP, Simpson JL, Felker RE *et al.* Transvaginal chorionic villus sampling using transabdominal ultrasound guidance. *Prenat Diagn* 1992; **12**: 229–234.
9. George K, George SS, James C *et al.* Transvaginal chorionic villus sampling – An alternative approach. *Aust N Z J Obstet Gynaecol* 1999; **39**: 487–489.
10. Firth HV, Boyd PA, Chamberlain PF, MacKenzie IZ, Morriss-Kay GM, Huson SM. Analysis of limb reduction defects in babies exposed to chorionic villus sampling. *Lancet* 1994; **343**: 1069–1071.
11. Maggio A, Giambona A, Cai SP, Wall J, Kan YW, Chehab FF. Rapid and simultaneous typing of hemoglobin S, hemoglobin C, and seven Mediterranean beta-thalassemia mutations by covalent reverse dot-blot analysis: Application to prenatal diagnosis in Sicily. *Blood* 1993; **81**: 239–242.
12. Rossetti LC, Radic CP, Larripa IB, De Brasi CD. Genotyping the hemophilia inversion hotspot by use of inverse PCR. *Clin Chem* 2005; **51**: 1154–1158.
13. Sellathamby S, Balasubramanian P, Sivalingam S *et al.* Developing an algorithm of informative markers for evaluation of chimerism after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2006; **37**: 751–755.
14. Brambati B, Oldrini A, Lanzani A, Terzian E, Tognoni G. Transabdominal versus transcervical chorionic villus sampling: A randomized trial. *Hum Reprod* 1988; **3**: 811–813.
15. Jackson LG, Zachary JM, Fowler SE *et al.* A randomized comparison of transcervical and transabdominal chorionic-villus sampling. *N Engl J Med* 1992; **327**: 594–598.
16. Liao C, Yang X, Li FT, Li J, Li DZ. The detection of aneuploidy and maternal contamination by QF-PCR in samples undergoing prenatal diagnosis for thalassemia in Southern China. *Eur J Obstet Gynecol Reprod Biol* 2009; **144**: 149–152.
17. Brambati B, Oldrini A, Ferrazzi E, Lanzani A. Chorionic villus sampling: An analysis of the obstetric experience of 1,000 cases. *Prenat Diagn* 1987; **7**: 157–169.
18. Scott F, Peters H, Boogert T *et al.* The loss rates for invasive prenatal testing in a specialised obstetric ultrasound practice. *Aust N Z J Obstet Gynaecol* 2002; **42**: 55–58.
19. Silverman NS, Sullivan MW, Jungkind DL, Weinblatt V, Beavis K, Wapner RJ. Incidence of bacteremia associated with chorionic villus sampling. *Obstet Gynecol* 1994; **84**: 1021–1024.
20. Fejgin M, Amiel A, Kaneti H, Ben-Nun I, Beyth Y. Fulminant sepsis due to group B beta-hemolytic streptococci following transcervical chorionic villi sampling. *Clin Infect Dis* 1993; **17**: 142–143.

21. Sidransky E, Black SH, Soenksen DM, Jones SL, Dorfmann AD, Schulman JD. Transvaginal chorionic villus sampling. *Prenat Diagn* 1990; **10**: 583–586.

22. Eisenberg B, Wapner RJ. Clinical procedures in prenatal diagnosis. *Best Pract Res Clin Obstet Gynaecol* 2002; **16**: 611–627.