Ultrasound Obstet Gynecol 2004; **23**: 482–485
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.1046

Early prenatal diagnosis by celocentesis

G. MAKRYDIMAS*, I. GEORGIOU*, I. BOUBA*, D. LOLIS* and K. H. NICOLAIDES†

**Department of Obstetrics and Gynaecology, Ioannina University Hospital, Ioannina, Greece and †Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK*

KEYWORDS: β-thalassemia; celocentesis; first trimester; Marfan syndrome; prenatal diagnosis

ABSTRACT

Background *Celocentesis is the ultrasound-guided aspiration of fluid from the extra-amniotic cavity at 7–8 weeks of gestation. This paper reports on the clinical application of celocentesis for early prenatal diagnosis.*

Methods *Celocentesis was successfully performed in nine pregnancies and 1–2 mL of fluid were obtained after one needle insertion. The indications were prenatal diagnosis of β-thalassemia or sickle cell disease (*n = *6), Marfan syndrome (*n = *1) and paternity testing (*n = *2). Molecular biological techniques were used to analyze the celomic fluid and this was successfully carried out in all cases.*

Results *In two cases pregnancy termination was performed at the request of the mother because in one case the fetus was found to have sickle cell anemia and in the second case paternity testing demonstrated that the father was not the woman's husband. In both cases the results were confirmed using the placental samples collected after pregnancy termination. In six of the seven pregnancies with desirable results, amniocentesis was performed at 16 weeks and the results were concordant with those obtained from celocentesis. All pregnancies were uneventful and resulted in the delivery of healthy and appropriately grown babies.*

Conclusion *Celocentesis may be a viable alternative to the currently used tests of chorionic villus sampling and amniocentesis. Copyright © 2004 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

In the first 10 weeks of pregnancy celomic fluid surrounds the amniotic sac. A sample of this fluid can be obtained by celocentesis, which involves the ultrasound-guided insertion of a needle into the extra-amniotic cavity through the vagina[1]. Molecular biological techniques can be used to analyze the DNA extracted from the cellular content of celomic fluid, making it feasible to carry out prenatal diagnosis of single-gene disorders, such as β-thalassemia, from as early as 6 weeks of gestation[2–5].

The established invasive techniques for prenatal diagnosis, namely chorionic villus sampling (CVS) and amniocentesis, cannot be undertaken safely before 10 and 15 weeks' gestation, respectively[6–9]. The need for earlier diagnosis essentially centers around two issues. First, earlier reassurance of parents that the fetus is normal and safer termination of pregnancy (TOP) in cases of affected fetuses. TOP can now be performed successfully in over 90% of cases with the use of misoprostol administered prior to the ninth week of gestation[10]. Second, the potential for intrauterine corrective therapy, such as stem cell transplantation, because after 11 weeks' gestation the fetus becomes immunologically intolerant[11].

Previous studies have examined the short-term safety of celocentesis, which was carried out prior to elective TOP. These studies have reported that celocentesis was not associated with significant feto-maternal hemorrhage or alteration in fetal heart rate[12,13]. Furthermore, in a study of women undergoing celocentesis 1–3 weeks before elective TOP we found that the rate of fetal death during this interval was only about 1% higher than in matched controls that had not undergone celocentesis[14].

In this paper we report our preliminary results on the clinical application of celocentesis for prenatal diagnosis.

METHODS

Celocentesis was performed at 7–8 weeks of gestation in the Department of Obstetrics and Gynaecology, Ioannina University Hospital, Ioannina, Greece for prenatal diagnosis of β-thalassemia or sickle cell disease ($n = 7$), Marfan syndrome ($n = 1$) and paternity testing

Correspondence to: Dr G. Makrydimas, Department of Obstetrics and Gynaecology, Ioannina University Hospital, Ioannina, Greece (e-mail: grmak@otenet.gr)

Accepted: 12 December 2003

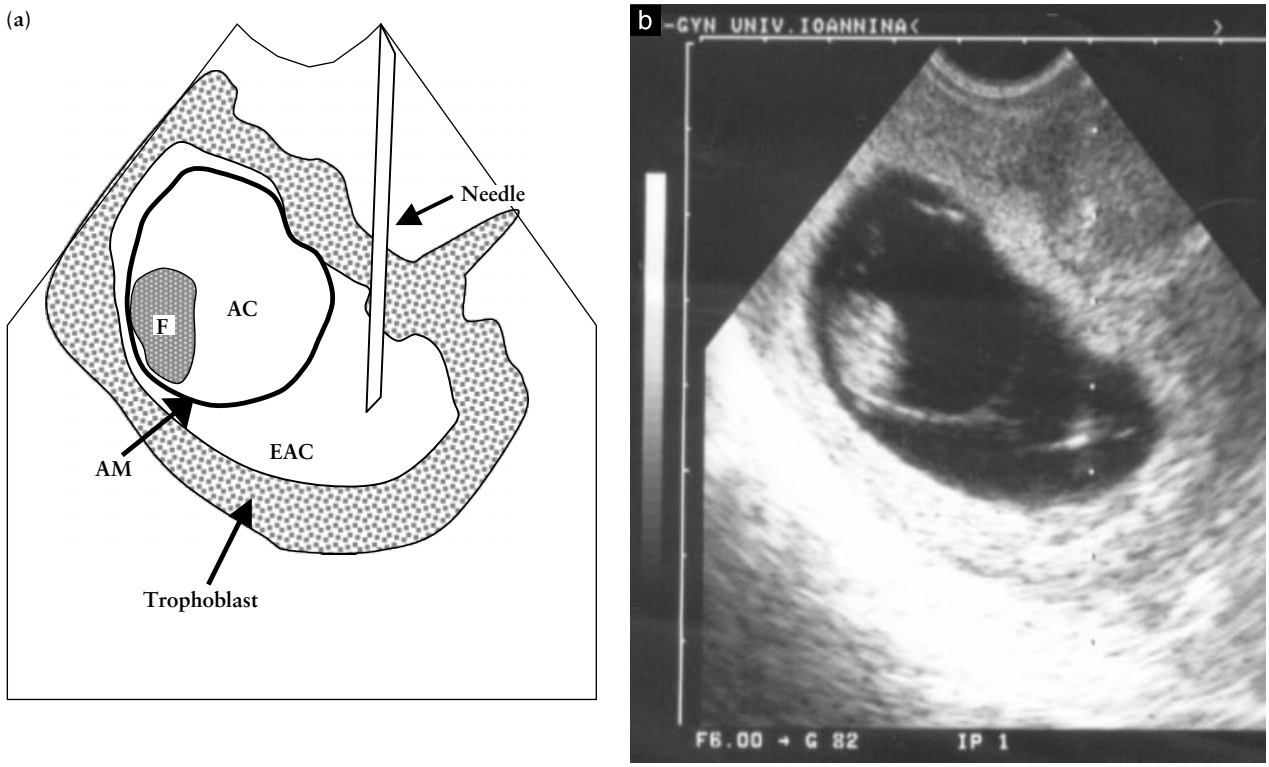

Figure 1 Schematic representation (a) and ultrasound image (b) of celocentesis. AC, amniotic cavity; AM, amniotic membrane; EAC, extra-amniotic cavity; F, fetus.

($n = 2$). Ethical approval was obtained from the hospital research committee and the women gave written consent. Celocentesis was successfully performed in all cases and 1–2 mL of fluid were obtained after one needle insertion.

Celocentesis

The external genitalia and the vagina were carefully cleansed with an antiseptic solution. Subsequently, transvaginal sonography using a 5-MHz ultrasound transducer (Toshiba SSA-220A, Toshiba, Tokyo, Japan) covered with sterile rubber was performed. The fetal crown–rump length and fetal heart rate were measured and the amniotic membrane, celomic space and yolk sac were identified. A 20-G needle was then introduced transvaginally into the celomic cavity through a guide attached to the transducer and fluid was aspirated (Figure 1). No local or general anesthesia was used and patients experienced only minimal discomfort. The first sample of 0.2 mL was discarded to avoid contamination with maternal tissue and a new syringe was then used to aspirate a further 1–2 mL of fluid, which were used for analysis.

Analysis of samples

Fetal DNA was extracted from celomic fluid cells after centrifugation and removal of the supernatant. The cell pellet was washed twice in a buffer containing 10 mM Tris-HCl, 50 mM potassium chloride and 1.5 mM magnesium chloride, resuspended in 25 μl of the same buffer and boiled for 10 min. Boiled samples were centrifuged briefly and 2–3 μl aliquots of the supernatant were used in the diagnostic polymerase chain reaction (PCR) experiments.

Genomic DNA from the parents was extracted from peripheral blood leukocytes by the standard saturated salt extraction method. Identification of parental mutations was performed by reverse dot-blot (β-Globin Strip-A, Vienna Lab, Vienna, Austria) allele-specific PCR with primers specific for the mutant and normal alleles and by denaturing gradient gel electrophoresis.

Fetal and parental DNA samples were analyzed in parallel. In the investigation of β-thalassemia/sickle cell disease, primers corresponding only to the parental mutations and β-globin gene fragments were used[2]. In addition, two highly informative polymorphic loci TPO and APOB variable number tandem repeats were used to examine for possible celomic cell contamination by maternal tissue and for paternity testing[3]. In the case of Marfan syndrome (OMIM 154 700) the father was the carrier of a known mutation[15]. We used restriction enzyme analysis by the endonuclease BspHI that recognizes the mutation and single-strand conformation polymorphism (SSCP) analysis with primers Fib15S and Fib15AS generating a 212 base pair fragment. Both SSCP and restriction enzyme analysis were used to diagnose the mutation in the celomic cells and the same methods were used to verify the results on cord blood DNA obtained at delivery (Figure 2).

Table 1 Indications for celocentesis and outcome of pregnancies

Indication	*GA at celocentesis (weeks)*	*CRL (mm)*	*Result*	*Outcome*	*GA at delivery (weeks)*	*Birth weight (g)*
β-thalassemia/sickle cell disease	8	16	Carrier	Live birth	37	2950
β-thalassemia	8	19	Normal	Live birth	36	2800
β-thalassemia	8	17	Carrier	Live birth	38	3150
β-thalassemia/sickle cell disease	7	11	Carrier	Live birth	39	2900
β-thalassemia	7	14	Carrier	Live birth	37	3100
Sickle cell disease	8	15	Affected	TOP	9	–
Paternity testing	8	15	Desired father	Live birth	38	3250
Paternity testing	7	11	Undesired father	TOP	8	–
Marfan syndrome	8	14	Normal	Live birth	40	3680

CRL, crown–rump length; GA; gestational age; TOP, termination of pregnancy.

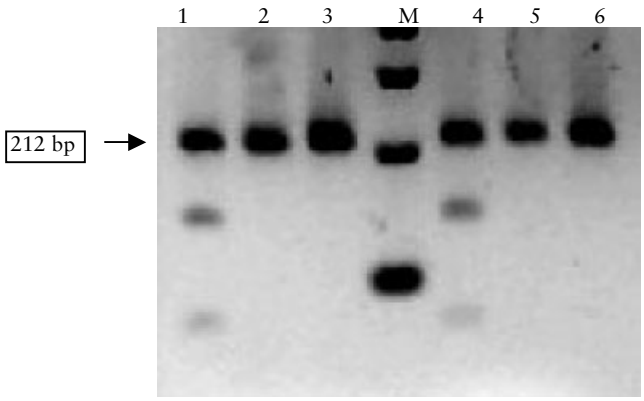

Figure 2 Restriction enzyme analysis by BspHI digestion. Lanes 1 and 4 represent the DNA from the father affected by the mutation G2001T, lanes 3 and 6 the DNA from the unaffected mother, lane 2 the celomic DNA sample and lane 5 the cord blood DNA collected at delivery. bp, base pairs; M, marker.

RESULTS

The results and pregnancy outcomes are summarized in Table 1. In two cases TOP was performed at the request of the mother because in one case the fetus was found to have sickle cell anemia and in the second case paternity testing demonstrated that the father was not the woman's husband. In both cases the results were confirmed using the placental samples collected after TOP. The seven pregnancies with desirable results were uneventful and they all resulted in the delivery of healthy and appropriately grown babies. In six cases amniocentesis was performed at 16 weeks' gestation and the results were concordant with those obtained from celocentesis. In the case of Marfan syndrome the results were verified on cord blood obtained at delivery.

DISCUSSION

The findings of this preliminary study demonstrate the feasibility of celocentesis in early prenatal diagnosis. In all cases celomic fluid was successfully obtained after one needle insertion and molecular biological techniques were used for the correct prenatal diagnosis of the conditions under investigation. Previous studies have reported limited success for traditional cytogenetic analysis using cells from the celomic fluid mainly because of poor growth in culture. However, as demonstrated in this study, celocentesis provides a sample of pure fetal cells for reliable DNA-based diagnosis.

The number of cases examined was too small for definite conclusions to be reached as to the safety of the technique. However, it is noteworthy that all seven continuing pregnancies resulted in the delivery of healthy and appropriately grown neonates. Similarly, there are a total of five cases from two previous reports on experimental celocentesis carried out before elective TOP in which the mother subsequently changed her mind and chose to continue with the pregnancy[14,16]. In four cases healthy and appropriately grown neonates were born at term but in one case there was preterm delivery at 25 weeks due to cervical incompetence[16]. The preliminary data provided by the present study suggest that celocentesis may be a viable alternative to the currently used techniques of amniocentesis and CVS for early prenatal diagnosis. However, definite conclusions on both the short- and long-term safety of the technique can only be drawn after the study of a much larger number of cases.

REFERENCES

1. Jurkovic D, Jauniaux E, Campbell S, Pandya P, Cardy DL, Nicolaides KH. Coelocentesis: a new technique for early prenatal diagnosis. *Lancet* 1993; **341**: 1623–1624.
2. Makrydimas G, Georgiou I, Kranas V, Zikopoulos K, Lolis D. Prenatal diagnosis of beta-thalassemia by coelocentesis. *Mol Hum Reprod* 1997; **3**: 729–731.
3. Makrydimas G, Georgiou I, Kranas V, Kaponis A, Lolis D. Prenatal paternity testing using DNA extracted from coelomic cells. *Fetal Diagn Ther* 2004; **19**: 75–77.
4. Jurkovic D, Jauniaux E, Campbell S, Mitchell M, Lees C, Layton M. Detection of sickle gene by coelocentesis in early pregnancy: a new approach to prenatal diagnosis of single gene disorders. *Hum Reprod* 1995; **10**: 1287–1289.
5. Findlay I, Atkinson G, Chambers M, Quirke P, Campbell J, Rutherford A. Rapid genetic diagnosis at 7–9 weeks' gestation: diagnosis of sex, single gene defects and DNA fingerprint from coelomic samples. *Hum Reprod* 1996; **11**: 2548–2553.
6. Nicolaides KH, Brizot M, Patel F, Snijders R. Comparison of chorionic villus sampling and amniocentesis for fetal karyotyping at 10–13 weeks' gestation. *Lancet* 1994; **344**: 435–439.

7. Sundberg K, Bang J, Smidt-Jensen S, Brocks V, Lundsteen C, Parner J, Keiding N, Philip J. Randomised study of risk of fetal loss related to early amniocentesis versus chorionic villus sampling. *Lancet* 1997; **350**: 697–703.
8. CEMAT Group. Randomised trial to assess safety and fetal outcome of early and mid-trimester amniocentesis. The Canadian Early and Mid-trimester Amniocentesis Trial (CEMAT) Group. *Lancet* 1998; **351**: 242–247.
9. 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.
10. Singh K, Fong YF, Dong F. A viable alternative to surgical vacuum aspiration: repeated doses of intravaginal misoprostol over 9 hours for medical termination of pregnancies up to eight weeks. *BJOG* 2003; **110**: 175–180.
11. Edwards RG, Jauniaux E, Binns RM, Layton M, Jurkovic D, Grillo TA, Campbell S. Induced tolerance and chimaerism in human fetuses using coelocentesis: a medical opportunity to avert genetic disease? *Hum Reprod Update* 1995; **1**: 419–427.
12. Makrydimas G, Lolis D, Georgiou I, Navrozoglou I, Nicolaides KH. Fetomaternal bleeding following coelocentesis. *Hum Reprod* 1997; **12**: 845–846.
13. Makrydimas G, Lolis D, Georgiou I, Skendou C, Nicolaides KH. Fetal heart rate following coelocentesis. *J Matern Fetal Med* 1997; **6**: 314–316.
14. Makrydimas G, Kaponis A, Skendou C, Lolis D. Short-term safety of celocentesis for the mother and the fetus. *Ultrasound Obstet Gynecol* 2002; **19**: 243–245.
15. Toudjarska I, Kilpatrick MW, Lembessis P, Carra S, Harlton G, Sisson ME, Black SH, Stern HJ, Gelman-Kohan Z, Shohat M, Tsipouras P. Novel approach to the molecular diagnosis of Marfan syndrome: application to sporadic cases and in prenatal diagnosis. *Am J Med Genet* 2001; **99**: 294–302.
16. Ross JA, Jurkovic D, Nicolaides K. Coelocentesis: a study of short-term safety. *Prenat Diagn* 1997; **17**: 913–917.

