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Randomized study of early amniocentesis versus chorionic villus sampling: a technical and cytogenetic comparison of 650 patients

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ABSTRACT

In a prospective randomized trial of 650 patients at 10–13 weeks' gestation, early amniocentesis was compared to chorionic villus sampling (CVS) for successful sampling and cytogenetic results and the speed with which results can be obtained. The indications for fetal karyotyping were advanced maternal age, parental anxiety or a family history of chromosomal abnormality in the absence of a balanced parental translocation. At early amniocentesis, 10 ml of amniotic fluid were successfully obtained in all 324 cases, in 320 (98.8%) at the first attempt and in four cases (1.2%) after two needle insertions. CVS provided sufficient material for cytogenetic analysis in 314 of 326 (96.3%) cases at the first attempt and in nine (2.8%) after two needle insertions; in three cases only maternal tissue was obtained and subsequently early amniocentesis was performed. Cell culture and chromosomal analysis were successful in 318 of 324 (98.1%) cases of early amniocentesis and in 321 of the 323 (99.4%) cases of CVS where villi were obtained. The median interval between sampling and successful result from cell culture was 12 days for early amniocentesis and 11 days for CVS. Mosaicism was detected in five cases of CVS but in none of the early amniocenteses; three of these cases were confirmed as true fetal mosaics. This study illustrates that early amniocentesis is a viable alternative to CVS for first-trimester fetal karyotyping. The results of the continuing prospective trial will establish the safety and cytogenetic accuracy of the two techniques.

INTRODUCTION

When amniocentesis for fetal karyotyping was first performed it was limited to 16 weeks' gestation onwards, because at earlier gestations there was a high failure rate in obtaining amniotic fluid[1]. Subsequently, the introduction of real-time ultrasonography for needle guidance made it possible to perform the technique at earlier gestations. However, doubts about the cellular content of the amniotic fluid in early pregnancy[2] and the introduction of chorionic villus sampling (CVS)[3] drew attention away from attempts at earlier amniocentesis.

CVS can be performed as early as 6 weeks' gestation[4] and cytogenetic results from direct preparation can be available within a few hours of sampling. However, the recent report[5] on the possible association between CVS at less than 10 weeks and fetal limb abnormalities is likely to confine its application to pregnancies beyond this gestation. Furthermore, reports of high false-positive[6,7] and, to a lesser extent, false-negative results[8] with direct preparation have led to guarded use of this method; for greater accuracy most laboratories now rely on culture before reporting results, which take 2–3 weeks from sampling. Recently, the European MRC trial has also demonstrated a significantly higher risk of spontaneous abortion after CVS than after second-trimester amniocentesis[7]. Despite these limitations, CVS has the advantage of providing prenatal diagnosis in the first trimester rather than at 18–20 weeks, as with traditional amniocentesis.

During the last 5 years, several studies of early amniocentesis have been reported (Table 1)[9–22] but no prospective comparison with CVS has been undertaken. Until such a study is completed the safety and cytogenetic accuracy of the procedure will not be known. On the assumption that the fetal loss rate from CVS is approximately 4%[6], and the difference between CVS and early amniocentesis is 1%, 6700 patients would need to be studied before it can be demonstrated that this difference is significant. This preliminary report of the first 650

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patients participating in such a prospective randomized trial of early amniocentesis and CVS compares the two procedures for the success rate in obtaining cytogenetic results and the interval between sampling and achieving results from cell cultures.

Table 1 Gestational distribution and total failure rate for published studies of early amniocentesis

		Weeks of gestation						*Failure rate*
First author	*Year*	*8*	*9*	*10*	*11*	*12*	*13*	(%)
Hanson[9]	1987				4	36	149	0.0
Miller[10]	1987					30	57	0.0
Cuoco[11]	1989			11	33	57	27	3.94
Lituania[12]	1989				13	35	56	0.0
Richkind[13]	1989				3	6	55	—
Rooney[14]	1989	1	9	8	7	7	6	21.0
Evans[15]	1989			5	10	21	48	3.5
Penso[16]	1990				9	179	177	1.7
Elejalde[17]	1990		3	6	18	77	98	—
Stripparo[18]	1990				11	38	82	7.5
Nevin[19]	1990		1	2	2	26	61	0.0
Klapp[20]	1990		12	12	9	13	6	0.0
Robello[21]	1991				5	23	49	0.0
Parker[22]	1991				2	2	10	14.3
Total		1	25	44	126	550	881	2.0
Present study				75	137	69	43	1.9

PATIENTS AND METHODS

Pilot study of early amniocentesis

In 56 patients undergoing elective termination of pregnancy at 8–13 weeks' gestation, 10 ml of amniotic fluid were successfully obtained by ultrasound-guided transabdominal amniocentesis in all cases. Culture and cytogenetic analyses were successful in all 28 cases where the fetal crown–rump length was > 37 mm (10 weeks) but in only 61% (17 of 28) of fetuses with crown–rump length of 24–37 mm (Figure 1).

Randomized trial

The entry criteria for the trial are: (1) a singleton pregnancy of 10–13 weeks' gestation and a minimum fetal crown–rump length of 38 mm, and (2) fetal karyotyping for low-risk indications, such as advanced maternal age, family history of chromosomal abnormality (in the absence of balanced parental translocation) and parental anxiety.

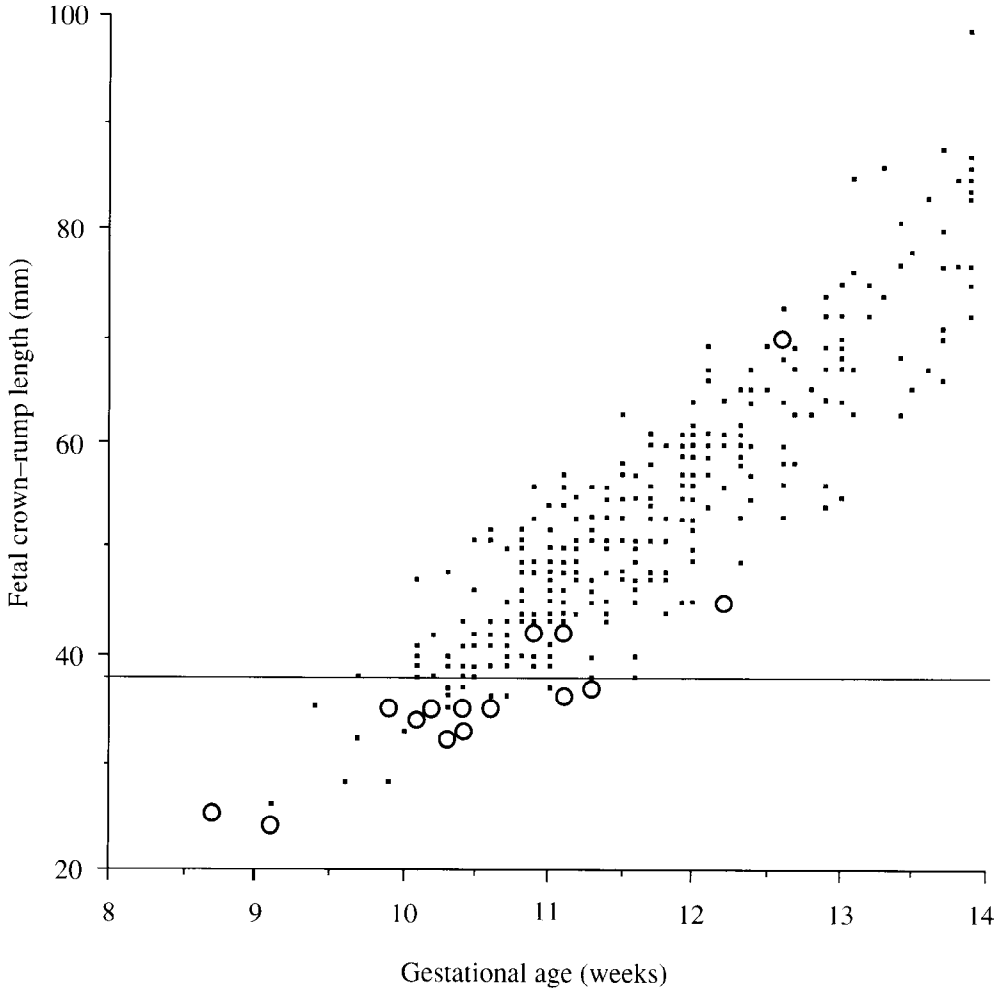

Figure 1 Relationship of fetal crown–rump length and gestational age in 380 pregnancies (including 56 from the pilot study) undergoing early amniocentesis, illustrating successful (·) and failed (o) cultures

Parents requesting fetal karyotyping are counselled about the three available options of:

(1) First-trimester CVS or early amniocentesis,

(2) Second-trimester amniocentesis, or

(3) Maternal triple biochemistry screening and ultrasound examination for chromosomal markers, followed by amniocentesis/cordocentesis in the minority of cases where the genetic risk is considered to be increased.

Those who request first-trimester diagnosis are given the options of having CVS or early amniocentesis, or to select one of two opaque envelopes; one contains a card with early amniocentesis written on it and the other a card with CVS written on it.

Both CVS and early amniocentesis are performed by transabdominal insertion of a 20-gauge needle into the uterus under continuous ultrasound control, using a free-hand technique. For CVS, the needle is guided into the placenta, taking care to avoid puncturing the amniotic sac. Chorionic villi are obtained using continuous suction provided by a 20 ml syringe held in an aspiration handle. The samples are examined and cleaned of blood and maternal tissue under a dissecting microscope and transported to the laboratory in culture medium. For early amniocentesis, the needle is guided into the amniotic sac through a placenta-free area of the uterus. Since at 10–13 weeks' gestation the amniotic sac is separated from the uterine wall, care must be taken to identify and puncture the amniotic membrane to ensure sampling of amniotic fluid rather than fluid from the extra-amniotic cavity. The first 1 ml of fluid is withdrawn and discarded, to avoid maternal cell contamination, and thereafter 10 ml are collected for culture.

In the laboratory, the CVS samples are examined under a dissecting microscope and suitable villi are dissected out and macerated finely. The amniotic fluid samples are aliquoted into three tubes, centrifuged, the supernatant discarded and the cell pellet, which is not always visible to the naked eye, is resuspended in culture medium. For both CVS and early amniocentesis, the cultures are grown in Ham's F10 medium supplemented with 2% Ultroser G and 5% fetal calf serum in a closed system at 37°C. Cultures are harvested using the suspension method[23], having previously been synchronized with a thymidine block. Routine karyotyping is performed on 15 mitoses from at least two tubes in both sample types.

RESULTS

CVS was performed in 326 patients and early amniocentesis in 324 in accordance with the randomization protocol (Figure 2). The median gestation of sampling was 11 weeks for both tests. Amniotic fluid was successfully obtained at the first attempt in 320 cases (98.8%) and after two needle insertions in four cases (1.2%). CVS provided sufficient material (> 5 mg wet weight) for cytogenetic analysis in 323 of the 326 cases, in 314 (97.2%) at the first attempt and in nine (2.8%) after two needle insertions. In three cases, the samples obtained, at the first attempt, were thought to be sufficient but subsequent examination in the laboratory revealed maternal tissue only.

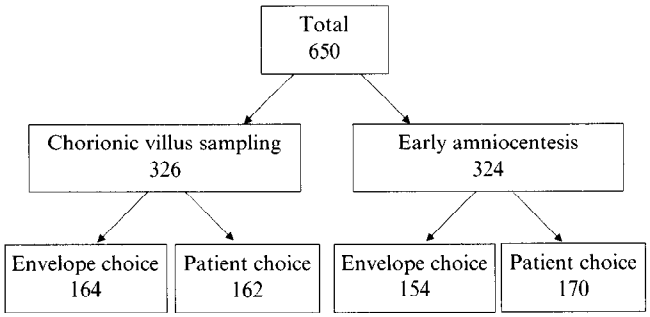

Figure 2 Distribution of patients between chorionic villus sampling and early amniocentesis, demonstrating method of choice

Table 2 Cytogenetic results of early amniocentesis and chorionic villus samples

Karyotype	*Early amniocentesis*	*CVS*	*Total*
Normal			
46,XX	150	143	293
46,XY	160	162	322
Abnormal			
Trisomy 21	4	4	8
Trisomy 18	2	3	5
Trisomy 13	—	2	2
Trisomy 22	—	1	1
47,XXY	1	1	2
47,XY,+ marker	1	—	1
Mosaic			
True mosaic	—	3	3
Confined placental mosaicism	—	1	1
Maternal cell contamination	—	1	1
Failed	6	5*	11
Total	324	326	650

* In three of these cases only decidual tissue was obtained

Cell culture and chromosomal analysis were successful in 318 of 324 (98.1%) cases of early amniocentesis; the karyotype was normal in 310 cases and abnormal in eight (Table 2). Culture failure occurred in three of the 75 cases at 10 weeks' gestation, two of 137 at 11 weeks' and one of 69 at 12 weeks' gestation. One had a repeat early amniocentesis, three a CVS, one a cordocentesis and one declined further investigation. Among those that had further sampling, four produced normal results and one yielded a double aneuploidy with trisomy 21 and a familial maternally-inherited marker chromosome (48,XY,+21,+mar).

In the CVS group, culture failure occurred in two of the 323 (0.6%) cases where a suitable sample was obtained. Both patients declined an offer for repeat testing, although one subsequently changed her mind and had cordocentesis at 20 weeks. In the three cases where the sample consisted of decidua only, an early amniocentesis was performed and normal results were obtained.

Table 3 Cytogenetic results of the five chorionic villus samples which presented diagnostic difficulties

Case	Cytogenetic findings: Karyotype	Tube 1	Tube 2	Tube 3	Cytogenetic follow-up	Interpretation
1	47,XX+8	93%	90%	100%	4%*	true mosaic
	46,XX	7%	10%	0%	96%*	
2	47,XX+21	10%	10%	—	7%† 30%‡	true mosaic
	46,XX	90%	90%	—	93%† 70%‡	
3	47,XXX	85%	77%	50%	7%‡	true mosaic
	46,XX	15%	23%	50%	93%‡	
4	46,XY	43%	19%	7%	100%*	maternal cell contamination
	46,XX	57%	81%	93%	0%*	
5	47,XX+2	100%	100%	100%	**	confined to placenta

* = Fetal blood sampling; † = amniocentesis; ‡ = skin biopsy; ** After counselling, the mother declined further tests; however, follow-up scans demonstrated normal fetal development

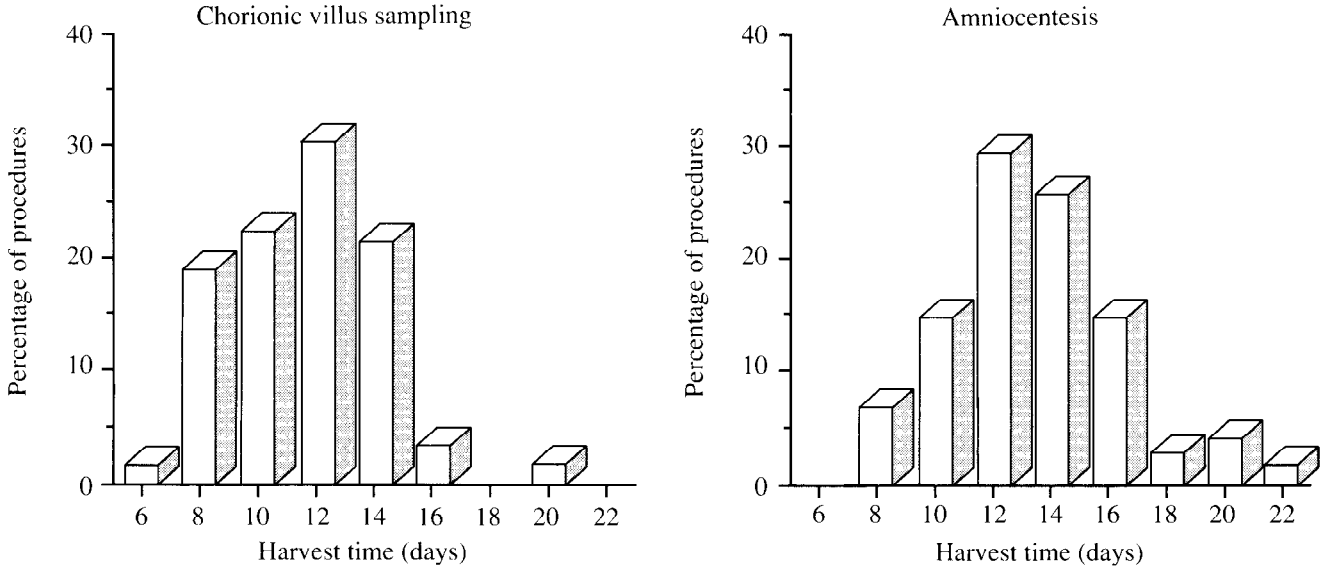

Figure 3 Distribution of harvest times for chorionic villus sampling and early amniocentesis

The cytogenetic results are shown in Table 2. There were no cases of mosaicism or pseudomosaicism in the early amniocentesis group. In contrast, there were three true mosaic results in the CVS group, two with autosomal trisomy mosaicism and one with 47,XXX mosaicism (Table 3). All three cases were shown to be apparently true fetal mosaics, and the karyotypes were confirmed on fetal blood or fetal skin following termination of pregnancy. In another case, all cells were trisomic for chromosome 2. This condition is known to be associated with confined placental abnormality and if real would have been incompatible with intrauterine life. Since the fetus was developing normally (as demonstrated by serial ultrasound scans), the parents received genetic counselling and chose not to have further invasive tests; the pregnancy is continuing.

The median interval between sampling and achieving cytogenetic results (Figure 3) for early amniocentesis was 12 (range 8–22) days and for CVS 11 (range 6–20) days.

DISCUSSION

This prospective randomized trial comparing early amniocentesis with CVS at 10–13 weeks' gestation has demonstrated high success rates in obtaining amniotic fluid and chorionic villi, using ultrasound-guided transabdominal insertion of a 20-gauge needle. The rates of 96% and 98% for successful sampling at the first attempt of CVS and early amniocentesis, respectively, compare favorably with the 69% rate for CVS and 94% rate of second-trimester amniocentesis reported in the European trial[7]. Furthermore, in our study the two procedures are performed in essentially the same way, the only difference being that CVS aims to avoid the amniotic sac and early amniocentesis to avoid the placenta. Therefore, when data from fetal loss rates are eventually available they are likely to reflect the inherent risk of sampling the different tissues, rather than the heterogeneous nature of the sampling techniques (including a wide range in

gestations for transabdominal or transcervical entry of aspiration cannulas, biopsy forceps or needles of variable sizes), which at least in part may have contributed to the findings of the Canadian, European and Danish randomized trials[6,7,24].

Successful cytogenetic results were obtained in 98% of cases for both early amniocentesis and CVS; insufficient tissue sampling in three and culture failure in two cases of CVS balanced the six culture failures in the early amniocentesis group. However, the high level of culture success with early amniocentesis is only achieved when the fetal crown–rump length is greater than 37 mm (Figure 1). In the majority of previous reports on early amniocentesis, the failure rates were similar to the present study (Table 1). The 21% failure rate in the study of Rooney *et al.*[14] can be attributed to the high proportion of cases sampled at less than 10 weeks' gestation.

The interval between sampling and obtaining cytogenetic results is similar for the two techniques. Although with CVS much faster results can be obtained by direct preparation, the consequence of such a policy would be an unacceptably high false-positive rate. The alternative of performing both direct preparation and culture doubles the laboratory workload[25] and therefore the cost, which is of critical importance for a test aimed at 5% of the pregnant population. Furthermore, to obtain sufficient material for both direct preparation and culture either a larger gauge needle or repeated insertions are required. In the present study, a single 20-gauge needle insertion was chosen because it is the least traumatic method, and therefore expected to be the technique with the lowest procedure-related risk.

The cytogenetic results obtained in the early amniocentesis group do not differ from those encountered in traditional amniocentesis; therefore it is appropriate to apply the extensive knowledge gained from over 25 years' experience with traditional amniocentesis to this new technique. There was initial apprehension that the small number of cells in early amniocentesis samples would allow an abnormal cell line to constitute a higher proportion of the total cell number and thus give an artificially high level of pseudomosaicism, but this has not been the case in our study. However, in four cases (1.2%) of CVS, where the initial cytogenetic result was mosaic and presented interpretation difficulties, a further prenatal test was required to elucidate the true fetal karyotype.

This study has demonstrated that early amniocentesis is a viable alternative to chorionic villus sampling for fetal karyotyping, in terms of successful sampling and cytogenetic results, as well as for the speed with which results can be obtained. Indeed, early amniocentesis has the advantage over chorionic villus sampling that the processing of samples requires less experienced laboratory staff, can be performed in batches and is less labor intensive. However, widespread application of early amniocentesis will depend on the safety and cytogenetic accuracy of the procedure, which will only be established with the result of a large prospective randomized trial of early amniocentesis and chorionic villus sampling. Until this information is available it is only appropriate to offer early amniocentesis within the confines of such a trial.

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Centers wishing to participate in this ongoing trial may obtain details and a protocol from the authors.