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# Why Cell Culture Is Successful after Early Amniocentesis

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**Abstract.** The current interest in early amniocentesis as a possible alternative to chorion villus sampling has driven many centres to attempt cell culture from first trimester amniotic fluid. This study examines the cellular content of 125 amniotic fluid specimens collected between 8 and 18 weeks of gestation and shows that there is a higher proportion of viable cells at the time of early amniocentesis than at traditional amniocentesis. Although there is a small increase in viable cell number from 8 to 18 weeks of gestation, this is not as dramatic as the exponential rise in total cell number seen over the same period. The similar concentration of viable cells in early pregnancy to that in the second trimester is sufficient to explain the unexpected culture success after early amniocentesis.

## Introduction

When genetic amniocentesis was introduced in the early 1970s the lack of ultrasound guidance limited it to a stage in pregnancy when the uterus was easily palpable (16–18 weeks). Despite the widespread use of ultrasound needle guidance in the 1980s, amniocentesis was still routinely performed within the same gestational range. There was no attempt at earlier amniocentesis, instead attention was diverted to first trimester chorion villus sampling (CVS) as an alternative technique. However, by the end of the decade the safety and diagnostic accuracy of CVS were being questioned [1, 2], and renewed interest focussed on earlier amniocentesis.

The major limitations to earlier amniocentesis were considered to be: access to the uterus, and an inadequate number of free cells in amniotic fluid to initiate a viable culture [3]. The former has been overcome with high resolution ultrasound for needle guidance, but what of the latter? In a prospective randomised study comparing amniocentesis and CVS at 10–13 weeks in our unit, am-

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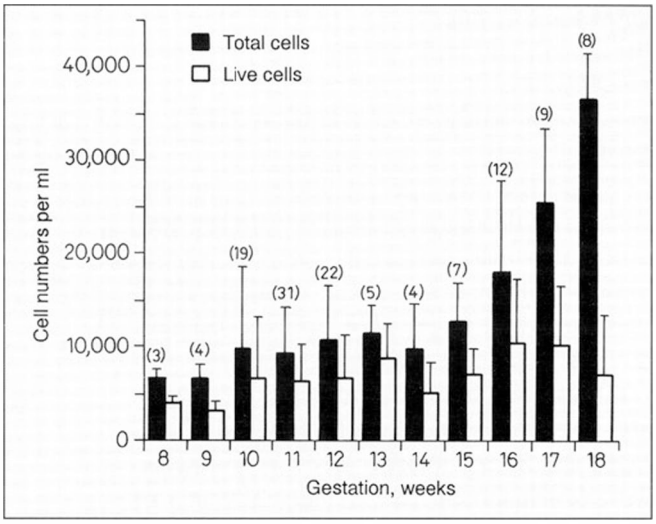


**Fig. 1.** Mean number of total and live cells per millilitre of amniotic fluid for each week of gestation. The error bars indicate one standard deviation of data. The numbers in parentheses are the number of samples.

niotic fluid cell culture was successful in 308 of 312 (98.7%) cases, when the fetal crown-rump length was > 37 mm (10 weeks and 1 day). Furthermore the harvest time was the same as in second trimester amniocentesis. This occurred in spite of the reported significant decrease in cell content of the amniotic fluid in the first trimester [4]. The aim of the present study was to investigate the apparent paradox of successful cell culture despite the dramatic fall in cell numbers within the liquor.

## Patients, Methods and Results

Amniotic fluid was collected by ultrasound-guided amniocentesis from 125 pregnancies at 8–18 weeks of gestation. In 78 cases the women were undergoing diagnostic amniocentesis for advanced maternal age and in 47 cases amniocentesis was performed immediately before elective termination of pregnancy. All patients gave their written consent and the protocol was approved by the hospital ethics committee. Gestational age was determined by the last menstrual period and confirmed by ultrasound measurement of fetal grown-rump length or biparietal diameter. Cytogenetic analysis demonstrated normal fetal karyotypes in all cases.

The vital stain trypan blue was used to identify live cells; 0.1 ml of amniotic fluid was incubated with 20 µl trypan blue at 37 °C for 5 min before being examined on a counting chamber (Neubauer haemocytometer). Total and viable cells were counted using light microscopy, viable cells excluded the dye and remained golden yellow in colour whilst dead cells stained blue [3]. All cell counts were performed twice and the mean number accepted.

The number of viable cells did not change significantly with gestation between 8 and 18 weeks, although the total number of cells increased exponentially over these gestations (fig. 1, $r = 0.91$, $p < 0.001$).

## Discussion

Although the total number of cells seen at the time of conventional amniocentesis (16–18 weeks) is significantly greater than at the stage of early amniocentesis (10–13 weeks), the number of viable cells is relatively simi-

lar at the two stages. This, in our opinion, is the reason why cell culture from early amniocentesis is as successful as traditional amniocentesis in the presence of such a low total cell count.

Since the exponential increase in the number of dead cells coincides with the gestation at which there is an icreasing contribution of fetal urine to the amniotic fluid volume [5], it is possible that the cellular increase is due to exfoliated cells from the genitourinary tract, the majority of which are not viable and therefore produce the rapid rise in cell number without adding to the live cell content. We are currently undertaking further investigation of the origin of cells in the amniotic fluid in early pregnancy to establish whether this is the case.

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