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Single uterine entry for genetic amniocentesis in twin pregnancies

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ABSTRACT

In 176 diamniotic twin pregnancies at 10–20 weeks of gestation, amniotic fluid for cytogenetic studies was successfully obtained from both sacs by the use of a single uterine entry. There were no cases of discordancy between sex at amniocentesis and birth. There were six pregnancies with fetal unbalanced chromosomal defects; in one pregnancy both fetuses were abnormal and in five pregnancies only one fetus was abnormal. The total fetal loss rate was 5.7% (20 of 352 fetuses), including six (1.7%) terminations or selective fetocides and 14 (4.0%) spontaneous deaths. In the 176 pregnancies there were five (2.8%) with no survivors, including one termination and four (2.3%) spontaneous miscarriages or intrauterine deaths. There are only two (1.1%) pregnancies in which amniocentesis could have contributed directly to the losses and therefore the procedure-related rate of fetal loss may be similar to that in singleton pregnancies. The median gestation at delivery was 37 (range 16–40) weeks and delivery before 32 weeks occurred in 9% of the pregnancies. The birth weight distribution was similar to that reported in singleton pregnancies. This study demonstrates that in twin pregnancies amniotic fluid for cytogenetic studies can be obtained successfully from both sacs by use of a single uterine entry. The risk of fetal loss from this procedure appears to be similar to that in singleton pregnancies.

INTRODUCTION

This study examined the efficacy and safety of fetal karyotyping by amniocentesis through a single uterine entry in 176 twin pregnancies. The data of previous studies of amniocentesis in twin pregnancies are summarized in Table 1. In all but one of the reports the procedure was performed by two uterine insertions of the needle and in at least six of the studies a dye was injected into the amniotic fluid of the first twin.

MATERIALS AND METHODS

During an 8-year period (January 1987 to February 1995), we performed amniocentesis at 10–20 weeks of gestation in 176 patients with twin pregnancies who were referred to the Harris Birthright Research Centre for Fetal Medicine. The indications were fetal karyotyping for advanced maternal age (n = 134), parental anxiety (n = 26) or increased first-trimester fetal nuchal translucency (n = 16).

Table 1 Studies of amniocentesis in twin pregnancies, providing data on complete pregnancy outcome, except the study of Buscaglia and colleagues[9], in which nine pregnancies were still ongoing at the time of publication. In that study, a single-needle technique was used in 48 of the 55 cases. In all other studies, a double-needle technique was used

			Gestation (weeks)			
Authors	*n*	*Years*	*Median*	*Range*	*Successful* (%)	*Spontaneous loss* (%)
Librach *et al.*[1]	70	1972–83	16	15–18	79	13.0
Tabsh *et al.*[2]	48	1977–83	?		98	9.4
Pijpers *et al.*[3]	83	1980–85	?	16–20	93	6.6
Pruggmayer *et al.*[4]	98	1982–89	17	15–20	?	10.7
Anderson *et al.*[5]	330	1969–90	?		?	5.0
Pruggmayer *et al.*[6]	529	1985–91	16	14–19	?	9.2
Wapner *et al.*[7]	81	1984–90	?	16–18	100	9.9
Ghidini *et al.*[8]	101	1987–92	17	14–20	?	3.5
Buscaglia *et al.*[9]	55	1985–94	?	14–18	100	4.4

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A careful ultrasound examination was first performed to determine the viability, size, sex and position of each fetus, the position of each placenta and umbilical cord and the placental attachment of the inter-twin membrane. The relationship between each amniotic sac and the cervix was used to classify the fetuses as twin 1 and twin 2. For amniocentesis, a single-operator technique was employed. The ultrasound transducer (curvilinear 3.5 or 5 MHz), held in the left hand, was used to identify a placenta-free area for entry of the needle, usually perpendicular to the inter-twin membrane. The appropriate site on the maternal abdomen was cleaned with antiseptic solution and a free-hand technique was used to guide a 20-gauge needle with a stylet into one of the amniotic sacs. The first 1 ml of fluid was discarded, to avoid possible contamination with maternal tissue, and the desired volume (1 ml per week of gestation) of amniotic fluid was aspirated into a new syringe. The stylet was replaced in the needle, which was then advanced through the inter-twin membrane into the second sac (Figure 1). The stylet was removed and then amniotic fluid was aspirated; the first 1 ml was discarded as previously to avoid possible contamination with fluid from the first sac. Local anesthesia, antibiotics or tocolytics were not given and the patients were advised to continue with their normal activity.

Patients with an abnormal result were counselled as to the available options of continuing with the pregnancy, selective fetocide or termination. The subsequent management of pregnancy and delivery was undertaken at the referring hospitals. Information regarding pregnancy outcome was obtained from the patients and the referring doctors.

RESULTS

The median maternal age was 38 (range 25–45) years and the median gestation at amniocentesis was 16 (range 10–20) weeks (Figure 2). The placentae were both anterior in 59 (34%) of the cases, both posterior in 60 (34%) and one anterior and the other posterior in 57 (32%). All pregnancies were diamniotic, but in 75 (43%) the twins were of the same sex and the placentae were on the same side of the uterus.

In all cases placental puncture was avoided and clear amniotic fluid was successfully obtained from both sacs through a single uterine entry. However, in two pregnancies there was culture failure for one of the samples (0.6%). The amniocenteses were performed at 16 and 18 weeks, respectively, and the parents were offered the option of cordocentesis for further karyotyping. They declined, because there were no ultrasonographic markers of chromosomal defects; in both cases the babies were live-born and are normal.

The karyotype was normal in 343 (97.4%) fetuses (183 male and 160 female) and there were no cases of discordancy between sex at amniocentesis and birth. There were six pregnancies with fetal unbalanced chromosomal defects; in one pregnancy both fetuses were abnormal and in five pregnancies only one fetus was abnormal (Table 2). In the first case termination of pregnancy was carried out, and in four of the other five cases selective fetocide was performed by intracardiac injection of potassium chloride. In the case affected by trisomy 18, the parents chose to continue with the pregnancy, which resulted in both babies being born alive, but the one with trisomy 18 died in the neonatal period.

In 161 of the pregnancies, both babies survived. In three cases, both babies died after spontaneous delivery at 16–24 weeks of gestation, following apparently uneventful amniocentesis (Table 3). In another pregnancy, an ultrasound scan at 11 weeks demonstrated monochorionic twins, the umbilical cords being very close to the inter-twin membrane. The twins were discordant in size and the bigger one had increased nuchal translucency thickness. Amniocentesis at 16 weeks demonstrated normal male karyotypes, but there was a sudden intrauterine death of both fetuses at 19 weeks. The post-mortem findings confirmed the suspected diagnosis of twin-to-twin transfusion syndrome.

In six pregnancies only one baby survived (Table 3). In four there was unexplained intrauterine death of one of the fetuses at 14–38 weeks of gestation, but the co-twin

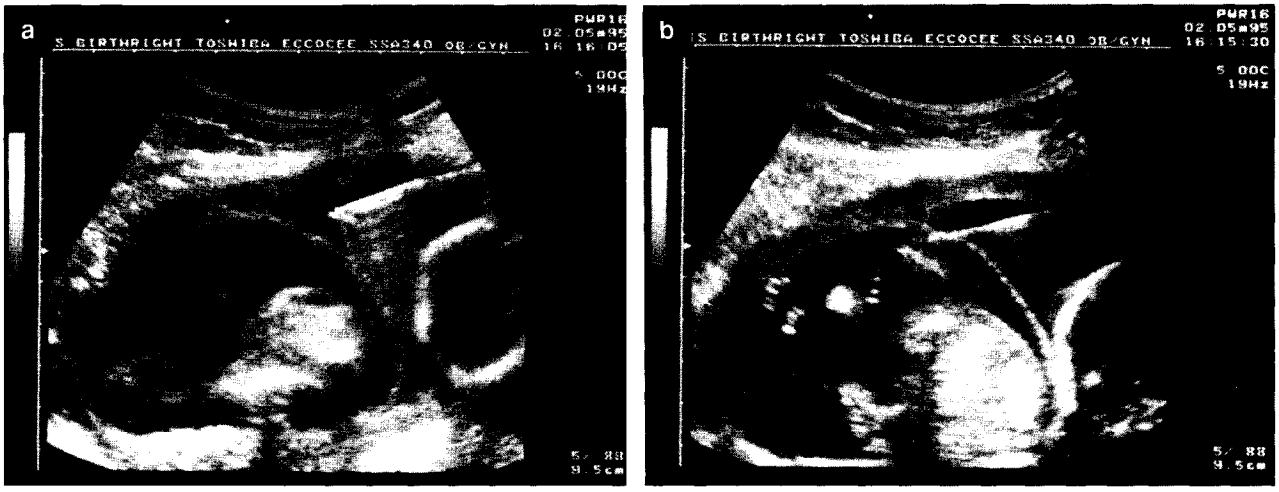

Figure 1 Amniocentesis needle sampling first sac (a), and clearly traversing the inter-twin membrane to reach the second sac (b)

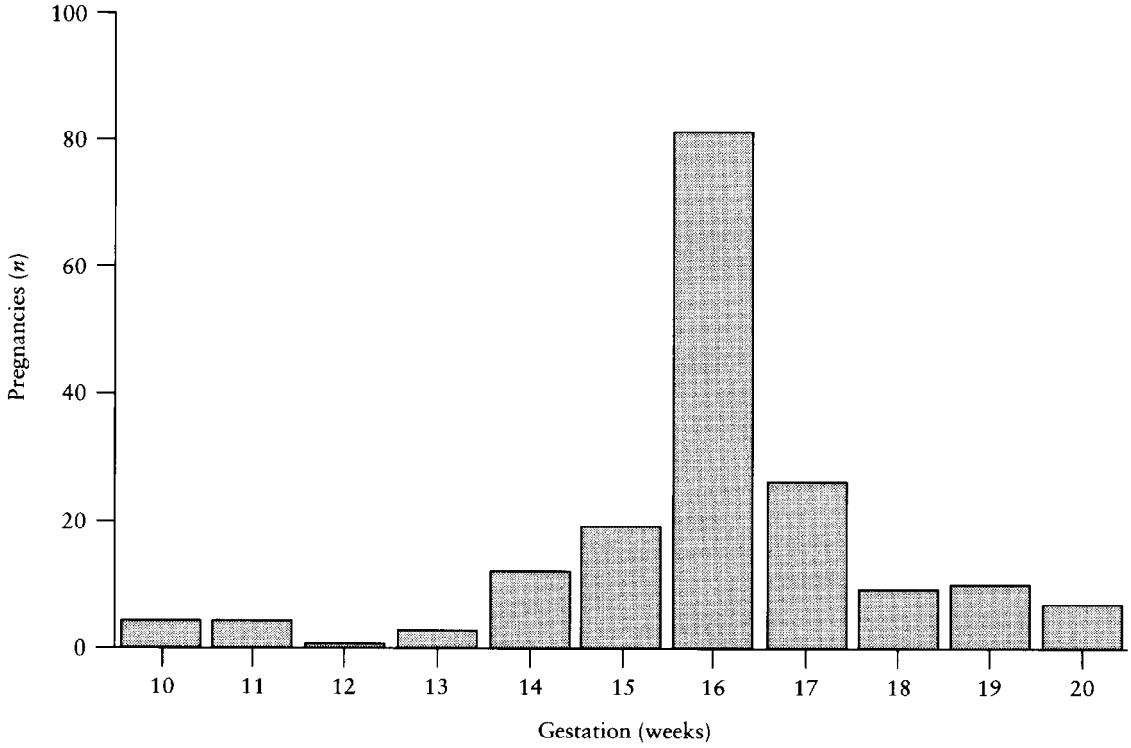

Figure 2 Frequency distribution of the gestation at amniocentesis in the 176 pregnancies

Table 2 Outcome of the six pregnancies with fetal chromosomal defects

Case	*Karyotype*	*Outcome*
1	47,XY + 21	termination of pregnancy
	47,XY + 21	termination of pregnancy
2	47,XX + 21	selective fetocide
	46,XY	live birth
3	47,XX + 21	selective fetocide
	46,XX	live birth
4	47,XY + 21	selective fetocide
	46,XY	live birth
5	47,XX + 18	neonatal death
	46,XY	livebirth
6	47,XXY	selective fetocide
	46,XY	live birth

survived. In another case the pregnancy was complicated by polyhydramnios at 28 weeks and ultrasound examination demonstrated arthrogryposis in the presenting twin that died at 32 weeks; there was spontaneous delivery at 33 weeks and the co-twin survived. Finally, one neonatal death occurred of a baby with trisomy 18. The parents had opted not to have selective fetocide performed.

The total fetal loss rate was 5.7% (20 of 352 fetuses), including the six (1.7%) terminations or selective fetocides and 14 (4.0%) spontaneous deaths. In the 176 pregnancies there were five (2.8%) with no survivors, including one termination and four (2.3%) spontaneous miscarriages or intrauterine deaths. The spontaneous fetal loss rate in the subgroup of 11 pregnancies that were sampled at 10–13 weeks was 9.1% (two fetal deaths), but this was not significantly different from the loss rate of 3.6% (12 fetal deaths) in the 165 that were sampled at 14–20 weeks ($\chi^2 = 0.49$, $p = 0.48$). Although in the subgroup of pregnancies with same-sex twins and placentae on the same side of the uterus the frequency of pregnancies with spontaneous fetal losses (six of 75 or 8%) was higher than in the other pregnancies (three of 101 or 3%), this difference was not significant ($\chi^2 = 1.33$, $p = 0.25$).

The median gestation at delivery was 37 (range 16–40) weeks and delivery before 32 weeks occurred in 16 (9%) of the 176 pregnancies (Figure 3). The birth weight distribution was similar to that reported in singleton pregnancies[10] (Figure 4). Apparently none of the survivors had talipes equinovarus. The only abnormality that was reported was tetralogy of Fallot in one of the babies; this was corrected surgically and the child, now 5 years old, is developing normally.

DISCUSSION

The findings of this study indicate that in twin pregnancies amniotic fluid for cytogenetic studies can be obtained successfully from both sacs by the use of a single uterine entry. This method necessitates a high degree of expertise in ultrasound scanning and needling techniques. It requires the accurate definition of the position of the two amniotic sacs and simultaneous visualization of both the needle and the inter-twin membrane. This ensures that both sacs are sampled and removes the need for instillation of dyes, which have been associated with adverse sequelae[6].

There were no cases of discordancy between sex at amniocentesis and birth. In the cases with discordancy between the twins for a chromosomal abnormality, the

Table 3 Gestation at amniocentesis, placental position and outcome of pregnancies with spontaneous losses

Case	*Gestation at amniocentesis* (weeks)	*Karyotype*	*Placenta*	*Outcome*
1	16	46,XX	anterior	miscarriage at 16 weeks
		46,XX	anterior	miscarriage at 16 weeks
2	16	46,XX	anterior	miscarriage at 21 weeks
		46,XX	anterior	miscarriage at 21 weeks
3	16	46,XY	posterior	intrauterine death at 19 weeks
		46,XY	posterior	intrauterine death at 19 weeks
4	18	46,XY	anterior	intrauterine death at 24 weeks
		46,XX	anterior	neonatal death at 24 weeks
5	12	46,XY	anterior	intrauterine death at 14 weeks
		46,XY	posterior	livebirth at 38 weeks
6	15	46,XY	posterior	intrauterine death at 32 weeks
		46,XY	posterior	live birth at 32 weeks
7	15	46,XX	posterior	intrauterine death at 32 weeks
		46,XY	posterior	live birth at 32 weeks
8	15	46,XX	posterior	intrauterine death at 37 weeks
		46,XX	posterior	live birth at 37 weeks
9	11	46,XY	anterior	intrauterine death at 38 weeks
		46,XY	anterior	live birth at 38 weeks
10*	19	47,XX + 18	posterior	neonatal death at 37 weeks
		46,XY	anterior	livebirth

*Case 5 from Table 2

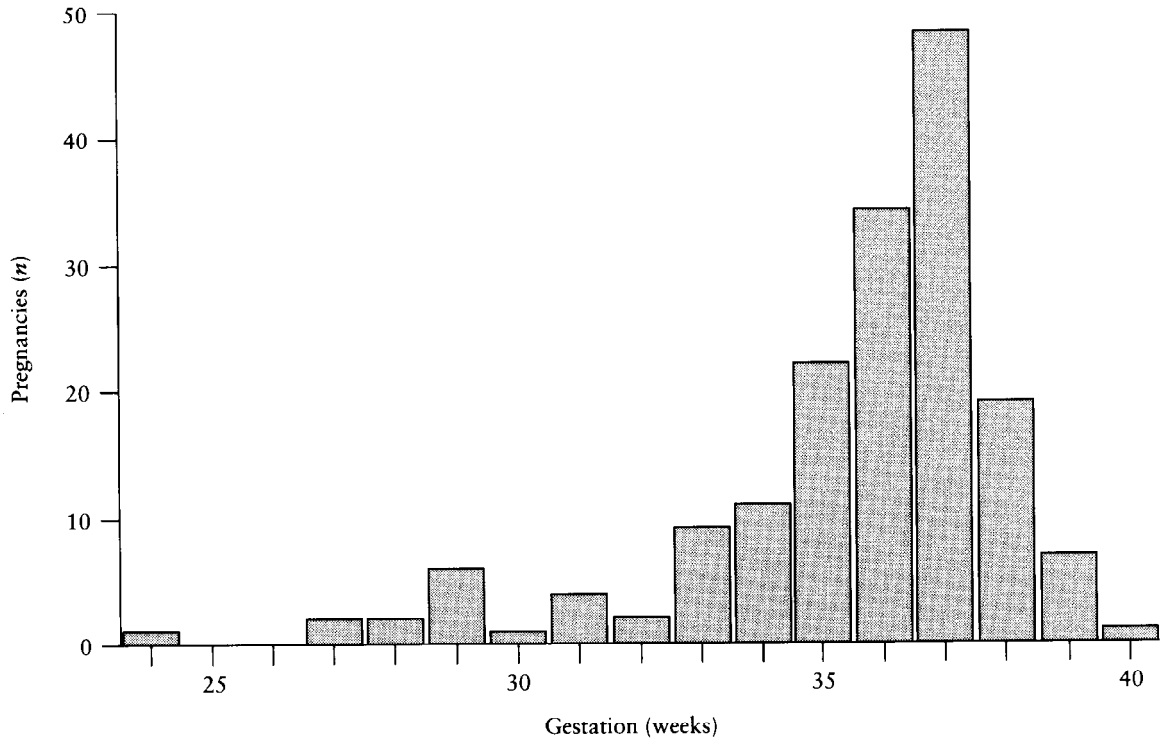

Figure 3 Frequency distribution of the gestation at delivery of live births

abnormal fetus was correctly identified at the time of selective fetocide. Such cases demonstrate the need for amniocentesis in twin pregnancies always to be performed in a specialist center, where selective fetocide can be undertaken. To avoid the potential disaster that fetocide of the normal twin is carried out, it is important that at the time of amniocentesis a careful ultrasound examination is performed to classify the fetuses as twin 1 and twin 2, depending on the relationship between each amniotic sac and the cervix. Discordancy in the sex of the fetuses is also useful in distinguishing between the normal and the affected one. It is also good practice that at the time of fetocide blood is taken for retrospective confirmation of the abnormal karyotype.

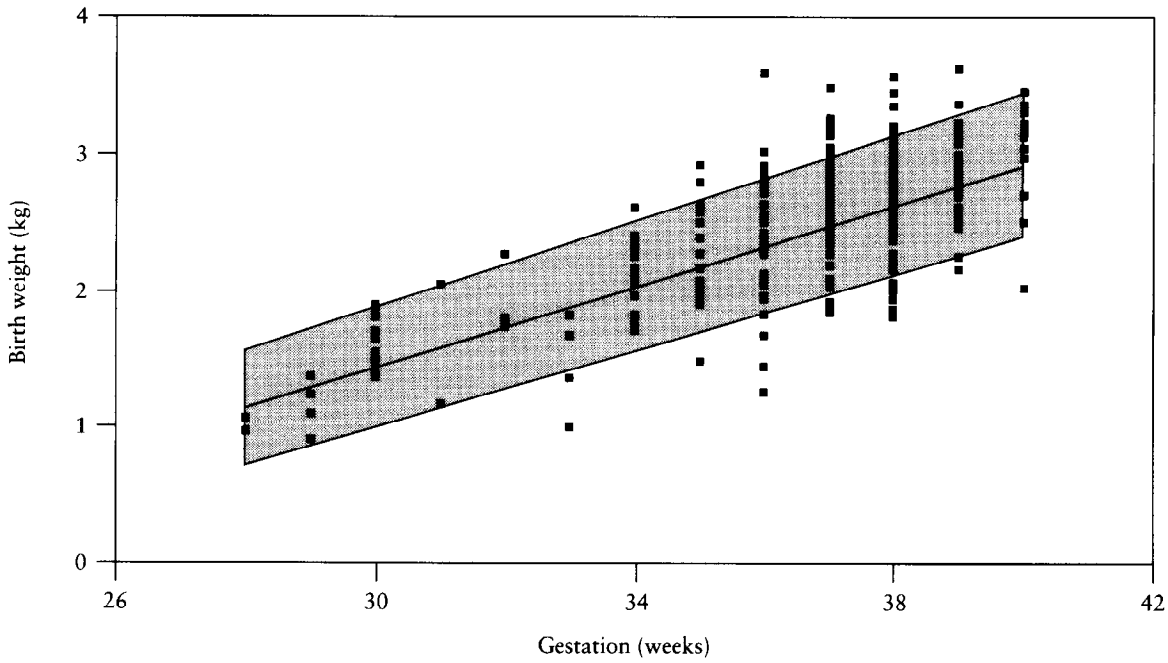

Figure 4 Individual birth weights for live births plotted on the normal range for gestation of singleton pregnancies (from reference 10)

Spontaneous death (intrauterine and neonatal) of both babies occurred in only 2.3% of pregnancies and the total rate of fetal death was 4.0%, which compares favorably with the results of previous studies (Table 1). The loss rate was 3.6% in the pregnancies that were sampled after 13 weeks. A study evaluating amniocentesis at 10–13 weeks of gestation in singleton pregnancies has demonstrated that the associated rate of fetal loss is much higher than with chorionic villus sampling[11].

It is unlikely that all fetal losses in our study were due to amniocentesis. For example, included in the total of spontaneous losses is the death of a neonate with trisomy 18. Additionally, in one case there was direct evidence of fetal death due to severe twin-to-twin transfusion syndrome. Similarly, in another pregnancy the cause of death of one of the fetuses at 32 weeks was a possible neuromuscular disorder leading to arthrogryposis and progressive hydrops. There were another three pregnancies with unexplained deaths of one of the fetuses during the third trimester, several months after apparently uneventful procedures. There were only two pregnancies in which miscarriage occurred within 5 weeks of the procedure, and in which amniocentesis could have contributed directly to the losses. The procedure-rate of fetal loss with single-needle amniocentesis in twins may therefore be around 1%, which would be similar to the rate of loss in singleton pregnancies. In a randomized study in singleton pregnancies comparing amniocentesis at 14–20 weeks of gestation vs. ultrasound scan alone, the rate of fetal loss in the amniocentesis group was 1.7% compared to 0.7% for the controls[12]. Although it is impossible to draw firm conclusions as to the safety of amniocentesis in twin pregnancies, in the absence of a randomized study comparing amniocentesis with no invasive intervention, it is unlikely that such a study will be undertaken. In a case–control study in which 101 twin pregnancies undergoing second-trimester amniocentesis were compared to 108 twin pregnancies undergoing ultrasound scans alone, the fetal loss rate in the two groups was similar (3.5% in the cases and 3.2% in the controls)[8].

The median gestation at delivery in this group of twin pregnancies was 37 weeks and the proportion that delivered at less than 32 weeks of gestation was 9%; this is similar to the 11.1% which occurred in the total of 88 848 twin pregnancies in the USA in 1992[13]. In addition, the birth weight distribution was similar to that reported in singleton pregnancies[10]. Although the number of cases is still relatively small for assessment of possible risks of amniocentesis-related congenital anomalies, it is of interest that there were no cases of talipes equinovarus. In a previous study of first-trimester amniocentesis in singleton pregnancies, the incidence of talipes was 1.63%[11], and in a second-trimester study the incidence was 0.8%[12].

In conclusion, amniocentesis through a single uterine entry allows successful sampling of both sacs, and the procedure-related loss rate may be similar to that of amniocentesis in singletons.

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