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Age-specific risk of fetal loss post second trimester amniocentesis: analysis of 5043 cases

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Objectives To assess the risk of fetal loss attributable to second trimester amniocentesis in singleton pregnancies through a cross-sectional study.

Methods Records of 5043 consecutive second trimester amniocentesis, performed by a single operator between 1997 and 2003, were analyzed. Fetal loss post amniocentesis was calculated by grouping pregnant women in age classes and assessing observed/expected (O/E) rate.

Results Total fetal losses were 40 (0.81%): 33 cases (0.67%) occurred before the 24th week, 37 cases (0.76%) before the 28th gestational week, and 3 cases (0.06%) after the 28th week of pregnancy. An age-dependent increase of the rate of fetal loss, not statistically significant (Chi-Square = 0.349, $p = 0.505$) was observed. The total O/E ratio values did not show any statistically significant risk (O/E ratio = 1.25, CI = 0.86–1.64). The analysis of the single age classes did not detect any statistical significance. The excess fetal loss rate associated with amniocentesis was 0.16%.

Conclusions No effect of the 2nd trimester amniocentesis was noted on fetal loss.

KEY WORDS: amniocentesis; fetal loss; maternal age

INTRODUCTION

Amniocentesis is an invasive prenatal diagnostic examination widely performed for screening fetal karyotypic abnormalities early in the second trimester of pregnancy. The number of procedures performed worldwide has seen a steep increase since its introduction: in USA the International Genetic Laboratory Directory indicated that in 1997 as many as 190 000 genetic amniocentesis were performed (Rebolloso and Knutsen, 1998), and in Italy the number of procedures has risen from 50 527 in 1995 to 101 000 in 2004 (Dallapiccola *et al*., 2006). By far the most common indication for the karyotypic analysis through amniocentesis is the increased maternal age, although an increasing number of younger women without any known genetic risk are presently undergoing this procedure (Dallapiccola *et al*., 2006).

Amniocentesis is not devoid of risks and their accurate estimate is essential for an informed decision: studies on the procedures conducted during the second trimester have found an increased rate of fetal loss, major orthopedic postural deformities, fetal injuries, respiratory problems in the neonate, preterm birth and low birth weight (NICHD National registry for amniocentesis study group, 1976; Medical Research Council of Canada, 1977).

Fetal loss is the most serious adverse procedure-related outcome: according to Seeds JW (2004), the origin of the commonly quoted 0.5% rate of pregnancy loss after amniocentesis, as it was recommended by the Centers for Disease Control and Prevention for amniocentesis counseling, is obscure (Centers for Disease Control and Prevention, 1995), since no source for this estimate was quoted and the number resulting differed from the only randomized prospective study reporting a 1% loss rate (Tabor *et al*., 1986).

In general, to calculate the fetal loss rate attributable to the procedure, the natural fetal loss rate during the 2nd trimester must be subtracted. Ultrasound-based studies have shown that the spontaneous fetal loss rate, assessed without considering maternal age and ethnicity, is approximately 1% after 16 weeks of gestation (Simpson, 1990). Recently, a large study performed in more than 250 000 women has shown that the risk of fetal loss in a second trimester serum screening population increased proportionally with maternal age and it was significantly correlated with ethnicity, with the highest rates among black women (Wyatt *et al*., 2005).

The aim of the present cross-sectional study, performed by a single operator, was to assess the risk of fetal loss attributable to amniocentesis in singleton pregnancies undergoing this procedure during the 2nd trimester.

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MATERIAL AND METHODS

Records of 5043 consecutive second trimester amniocentesis, performed between 1997 and 2003 by a single operator, were reviewed for indication, number of needle insertions, gross appearance of amniotic fluid, fetal karyotype and pregnancy outcome.

All amniocentesis were performed under ultrasound guidance in fetuses with a biparietal diameter (BPD) between 30 and 39 mm (corresponding in our biometrical charts, to 15^{+0} and 17^{+4} weeks of gestational age, respectively), with 21-gauge needles for the initial 2800 procedures, and with 22-gauge needles for the last 2243. The examination was performed under the following indications: advanced maternal age (57.3%), psychological indication (35%), abnormal biochemical test (3.5%), positive obstetric history (2.1%), abnormal nuchal translucency (1.3%), and abnormal sonographic findings (0.7%).

Twelve women needed to repeat the procedure: in six cases (0.1% of the total) due to a sampling failure, and in six others for a failure in the amniocytes culture. In 67 (1.3%) cases the fluid was murky/brown and in 45 (0.9%) cases the fluid was bloody.

The pregnancy outcome was determined by telephone interviews with the women. When a case of fetal loss was referred, the clinical chart was controlled: pregnancy outcome was categorized as elective abortion or spontaneous fetal loss, and was defined as spontaneous miscarriage (ICD-9 code 632, and 634.0–634.9) or intrauterine fetal demise (IUFD; ICD-9 code 656.4), as classified by the ICD-9 coding system. The spontaneous fetal loss rate was calculated as a percentage of fetal loss in all pregnant women (including pregnancies ending as miscarriage, IUFD, preterm pregnancy termination, stillbirth, and livebirth) for which a follow-up was available.

Exclusion criteria were fetal karyotypic abnormalities and malformations for which an elective abortion was decided. All the other malformations and karyotypic abnormalities were included in the study. All women with uterine malformations, obstetric history of recurrent abortions, or first trimester bleeding in pregnancy were included in the study.

All women were of Caucasian ethnicity and were divided into four age categories (<30, 30–34, 35–39, >39 years). Mantel–Haenszel Chi-square test for trend was used for the analysis of linear trend of fetal loss according to age groups. The spontaneous fetal loss rate was calculated from a reference population consisting of 250 011 pregnancies described by Wyatt *et al*. (2005), in which the outcomes according to the ICD-9 coding were available for 94.6% of the screened cases. In the study by Wyatt *et al*., all pregnancies with fetal chromosomal anomalies, fetal structural abnormalities, insulin dependent diabetes mellitus IDDM and multiple pregnancies were excluded. The authors adjusted the results for fetal loss due to amniocentesis according to the following function: number of screening positive women ×70% (uptake rate of amniocentesis) x 0.3% (estimated amniocentesis-related fetal loss rate from the literature) (Blackwell *et al*., 2002; Scott *et al*., 2002). The data, through a direct standardization for age and race to our population, constituted the expected cases of spontaneous fetal loss for every age group. The comparison between our observed cases and the expected was expressed by the observed/expected rate (O/E rate).

RESULTS

A total of 5043 women with singleton pregnancies underwent amniocentesis between January 1, 1997 and December 31, 2003; of these, 50 women (1% of the total) were lost to follow-up and were excluded. Fetal karyotypic abnormalities were observed in 95 cases (1.9%) (Table 1) and 43 of them decided to terminate the pregnancy. Moreover, 33 pregnancies were interrupted due to the finding of a major malformation. After exclusion of these two latter groups (76 pregnancies in total), the study population consisted of 4917 subjects. Among them, no lethal abnormalities were present.

Table 1—Summary of the fetal karyotypic abnormalities found in the study population

Fetal karyotype abnormalities	N°
Trisomy 21	50
Trisomy 18	8
Trisomy 13	2
Trisomy 9	1
47 XXX	2
47 XXY	2
47 XYY	1
45 X	2
69 XXX	2
Mosaicism	8
Unbalanced translocation	1
Chromosome inversion	6
Balanced translocation	10
Total	95

Table 2—Timing of fetal losses following amniocentesis in the study population

Weeks after amniocentesis	Fetal loss	
	No.	%
0	2	5.3
1	2	5.3
2	3	7.9
3	4	10.5
4	7	18.4
5	5	13.5
6	4	10.5
7	2	5.3
8	2	5.3
9	2	5.3
10	2	5.3
13	2	5.3
20	1	2.6
23	1	2.6
24	1	2.6
Total	40	100

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Table 3—Observed *versus* expected fetal losses after amniocentesis in the study population

Age classes	Number of cases		Observed fetal losses		Rate of spontaneous fetal loss in Caucasians (Wyatt *et al*. 2005)	Expected spontaneous fetal loss in the study population[a]		
	No.	%	No.	%	%	No.	O/ER	CI
25–29	572	11.6	4	0.69	0.33	2	2	0.04–3.96
30–34	1463	29.7	11	0.75	0.43	6	1.83	0.75–2.92
35–39	2380	48.4	20	0.84	0.71	17	1.18	0.66–1.69
>39	502	10.2	5	0.99	1.31	7	0.71	0.09–1.34
Totals	4917	100	40	0.81	0.65	32	1.25	0.86–1.64

C.I., confidence interval; O/E R, observed/expected rate.
[a] The data are calculated by dividing the number of cases (column 2 in the table) by the rate of spontaneous fetal loss (column 6 in the table) × 100.

Total fetal losses were 40 (0.81% of the total): 33 cases (0.67%) occurred before the 24th week, 37 cases (0.76%) before the 28th gestational week, and 3 cases (0.06%) after the 28th week of pregnancy.

Table 2 describes the timing of the spontaneous fetal loss, showing a peak at the 4th week following amniocentesis (from 19^{+0} to 21^{+0} weeks of gestational age). Table 3 reports the observed age-specific fetal loss rates after amniocentesis: an age-dependent increase can be observed, although Chi-square test for linear trend reveals that such an increase is not statistically significant (Chi-square = 0.349, $P = 0.505$).

Total observed fetal loss in our population was 0.81%. The excess fetal loss rate associated with the procedure was 0.16% of the study population (8 cases). The total O/E ratio values (Table 3) did not show any statistically significant risk (O/E ratio = 1.25, CI = 0.86–1.64). Also the analysis of the single age classes did not detect any statistical significance (Table 3).

DISCUSSION

In our study population the excess fetal loss rate associated with amniocentesis was 0.16%, with a total of 40 observed fetal loss *versus* the 32 expected from the natural fetal loss rate. The resultant O/E ratio was 1.25 (CI = 0.86–1.64) (Table 3): no statistically significant effect of 2nd trimester amniocentesis on the fetal loss was seen.

By assuming as expected the fetal loss rate percentages reported by Wyatt *et al*. (2005) after standardization for age and ethnicity *versus* our population (0.65%), the obvious bias of comparing different populations with different obstetric risk factors was probably reduced but not completely eliminated. On the other hand, a completely randomized trial of women at high risk for chromosomal abnormalities as a study population is not ethically acceptable, in a double blind prospective study. The only double blind randomized prospective trial was conducted in 1986 by Tabor *et al*., reporting a spontaneous abortion rate of 1.7% in the study group after amniocentesis and 0.7% in the control group (relative risk 2.3). This study was heavily criticized as it was carried out among young low-risk women (maternal mean age 28.6 ± 2.7), not representing most of the subjects undergoing this diagnostic procedure (Seeds, 2004). Seeds (2004) analyzed 14 studies with a total number of 34 144 amniocentesis performed under ultrasound guidance. These studies arrived at an heterogeneous conclusion regarding the pregnancy loss rates attributable to the procedure: the mean fetal loss rate after amniocentesis, observed before the 28th week of gestational age was 1.4%, with a range among the reported authors between 0.8% (Eiben *et al*., 1997) and 2.2% (Andreasen and Krisoffersen, 1989). Five of these 14 studies had a control group with a mean fetal loss rate of 1.08% with a range of 0.35–1.5% (Farahani *et al*., 1984; Tabor *et al*., 1986; Andreasen and Krisoffersen, 1989; Tongsong *et al*., 1998; Antsaklis *et al*., 2000).

One of the most important variables that can modify the natural fetal loss rate is represented by the maternal age, as reported in a number of studies (Harlap *et al*., 1980; Kline and Stein, 1984; Petitti, 1987; Risch *et al*., 1988; Berkowitz *et al*., 1990; Coste *et al*., 1991; Fretts *et al*., 1995; Nybo Andersen *et al*., 2000), in which an older maternal age was associated with a higher risk, both as natural fetal loss rate, and loss after amniocentesis. The natural fetal loss rate was recently assessed in the study of Wyatt *et al*. (2005), conducted on 250 011 women, reporting an inverse relationship between the risk of spontaneous fetal loss and maternal age, until women reached 27 years of age, when the lowest fetal loss rate was observed. For women >28 years, a proportional increase in the risk of spontaneous fetal loss was found with maternal age, to reach the highest risk category in women >44 years (with about 3%) (Wyatt *et al*., 2005). Also, fetal loss rates varied substantially among women of different ethnicity, with black women having the highest (1.80%). Fetal loss rate was 0.47% for Caucasians, the only ethnicity represented in our study population.

In our study, an increasing rate of spontaneous fetal loss with age was observed (0.69 to 0.99% from the youngest to the oldest age category) (Table 3), although not reaching statistical significance. Notably, the abortion rate had a peak between 19^{+0} and 21^{+0} weeks of gestational age (Table 2): these results are in line with previous reports in which the spontaneous fetal

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loss was determined and a peak was observed at about 20^{+0} weeks (Ancel *et al*., 2000; Hoesli *et al*., 2001).

In conclusion, we suggest that the conventionally stated pregnancy loss rate of 0.5% is no longer appropriate in experienced hands; in our data the excess fetal loss rate associated with the procedure was 0.16%, probably due to the increased experience of the operator and to the improved ultrasound technology, confirming that 'surely it is illogical to counsel the same 0.5% risk offered a quarter century ago when ultrasound was not available' (Elias and Simpson, 2004).

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