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Prenatal genetic testing by amniocentesis appears to result in a lower risk of fetal loss than chorionic villus sampling in singleton pregnancies achieved by intracytoplasmic sperm injection

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Objective: To compare pregnancy outcome after prenatal genetic testing by chorionic villus sampling (CVS) or amniocentesis in singleton pregnancies achieved by intracytoplasmic sperm injection (ICSI).

Design: Retrospective analysis.

Setting: Tertiary referral center.

Patient(s): Eight hundred twenty-eight patients with singleton gestations achieved by ICSI.

Intervention(s): Midtrimester amniocentesis (685 patients) and first-trimester CVS (143 patients).

Main Outcome Measure(s): Fetal loss rate, preterm delivery rate, and proportion of babies born with low or very low birth weight.

Result(s): A significant difference was observed in fetal loss rate between CVS and amniocentesis (3.7% vs. 0.9%, respectively). On the other hand, a similar preterm delivery rate was present between the two methods (11.2% vs. 12.4%, respectively). No significant difference was observed between amniocentesis and CVS in the proportion of babies with birth weight of either $<$1,500 g (1.8% vs. 3.8%, respectively) or between 1,500 and 2,500 g (8.2% vs. 4.6%, respectively).

Conclusion(s): Amniocentesis appears to result in a lower risk of fetal loss as compared with CVS in patients with a singleton pregnancy achieved by ICSI. (Fertil Steril® 2003;79:374–8. ©2003 by American Society for Reproductive Medicine.)

Key Words: Chorionic villus sampling, amniocentesis, ICSI, fetal loss, prematurity, birth weight

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Compared with the normal population, children born after intracytoplasmic sperm injection (ICSI) carry a higher risk of de novo sex chromosomal abnormalities and of inherited preexisting structural chromosomal aberrations (1, 2). Therefore, it is important to counsel couples who achieve pregnancy by ICSI about prenatal genetic testing.

The choice of prenatal diagnosis method depends on several parameters. Among these, of great importance is the risk of fetal loss that each procedure carries. In ICSI pregnancies, it has been shown that prenatal genetic testing by midtrimester amniocentesis in singleton pregnancies and by CVS in twin pregnancies does not result in an increased risk of fetal demise (3). However, no comparative data on the risk of fetal loss exist between CVS and amniocentesis in singleton ICSI gestations. Such information could help couples treated successfully by ICSI to choose a prenatal diagnosis method.

The purpose of the current study was to compare pregnancy outcome after CVS or amniocentesis in patients who achieved a singleton pregnancy after ICSI.

MATERIALS AND METHODS

The obstetric outcomes of 685 singleton ICSI pregnancies in which amniocentesis was performed were compared with those of 143 singleton ICSI pregnancies in which CVS was

carried out. Prenatal genetic testing was part of a prospective ICSI follow-up study at the Center for Reproductive Medicine of the Dutch Speaking Brussels Free University (4) between the years 1992 and 2000. The current study was approved by our institutional review board.

The methods followed for amniocentesis and CVS have been described in detail elsewhere (3, 5). In brief, during amniocentesis, a 20-mL sample of amniotic fluid was aspirated with a 22G spinal needle. Chromosome preparations were obtained from cultured amniocytes according to a modified technique (6). On the other hand, CVS was performed transcervically or transabdominally using a double-needle system (outer needle, 18G; inner needle, 20G). Short- and long-term preparations from cultured chorionic villi were obtained by procedures reported elsewhere (7, 8). Both amniocentesis and CVS were carried out by the same two experienced operators.

Obstetric outcomes after prenatal diagnosis were assessed by the frequencies of preterm delivery (<37 weeks), low birth weight (<2,500 g), very low birth weight (<1,500 g), and fetal loss. Patients who had two consecutive prenatal tests to confirm previous karyotyping were not included in the current study.

Normally distributed continuous variables (Kolmogorov-Smirnov Test with Lilliefors correction) were tested with the *t* test for independent samples. Fisher's exact test was used to analyze nominal variables in the form of frequency tables, and binary logistic regression, to identify the effect of different parameters on fetal loss risk. All tests were two-tailed, with a confidence level of 95% ($P<.05$). Values are expressed as mean ± standard error.

RESULTS

Amniocentesis and CVS were performed at mean gestational ages of 15.6 ± 0.5 weeks and 11.1 ± 0.8 weeks, respectively. Maternal age was lower ($P<.001$, *t* test) in the amniocentesis group (32.4 ± 0.2 years; range, 20–47 years) as compared with in the CVS group of patients (33.8 ± 0.4 years; range, 22–50 years).

Cytogenetic Abnormalities

A lower percentage ($P<.001$, Fisher's exact test) of cytogenetic abnormalities was present after amniocentesis as compared with CVS (2.9% vs. 9.8%, respectively; see Table 1). No significant difference was observed overall in the proportion of cytogenetic abnormalities detected in women <35 years of age and ≥35 years of age (3.8%, 22/579 vs. 4.8%, 12/249, respectively). All cases of autosomal trisomies occurred in women ≥35 years of age (mean, 38.8 ± 0.8 years).

Terminations of Pregnancy

Thirteen pregnancies were terminated after prenatal genetic testing in both groups. As shown in Table 2, a pregnancy was terminated because of detection of a cytogenetic abnormality after prenatal genetic testing in eight patients (CVS: 6; amniocentesis: 2). Moreover, five terminations were carried out because of abnormal ultrasound findings (amniocentesis: 2; CVS: 1), severe maternal hypertension (amniocentesis: 1), and the presence of fragile-X (CVS: 1). The proportion of terminations in the CVS group (5.6%, 8/143) was significantly higher ($P\leq.001$, Fisher's exact test) than that in the amniocentesis group (0.7%, 5/685).

TABLE 1

Cytogenetic diagnosis after CVS and amniocentesis in singleton pregnancies achieved by ICSI.

	Prenatal procedure	
Diagnosis	Amniocentesis	CVS
46, XY	368	55
46, XX	293	73
Cytogenetic abnormality, [a]n (%)	20 (2.9)	14 (9.8)
Failure	4	—
Missing results	—	1
Total	685	143

[a] $P<.001$ Fisher's exact test (95% CI of the difference between CVS and amniocentesis: 1.8–11.9%).

Kolibianakis. ICSI outcome after CVS and amniocentesis. Fertil Steril 2003.

The above terminations were not considered in the analysis of fetal loss risk between CVS and amniocentesis. However, an additional interruption of pregnancy, after premature rupture of membranes at 16 weeks of gestation in the CVS group, was considered as an early fetal loss. This was due to the fact that the pregnancy would have aborted in any case without intervention.

Comparison of Fetal Loss Risk Between CVS and Amniocentesis

Overall, the analysis of fetal loss risk between the two methods included 680 cases of amniocentesis and 135 cases of CVS. A significant difference ($P\leq.02$, Fisher's exact test) was observed in fetal loss rate between CVS (3.7%; 5/135) and amniocentesis (0.9%; 6/680; odds ratio, 4.3; 95% confidence interval [CI], 1.3–14.4). The absolute risk reduction was 2.8%, and the number needed to treat was 35. This means that for every 35 patients treated with midtrimester amniocentesis, one extra fetal loss is prevented. Ten of 11 fetal losses occurred in patients with a normal result after prenatal genetic testing, whereas a late fetal loss (38.2 weeks) occurred in an ICSI pregnancy in which the karyotype was 46, XX/47, XXX. No fetal losses occurred in the CVS group before 16 weeks of gestation.

Binary logistic regression, with fetal loss as a dependent variable and maternal age, type of sampling procedure (amniocentesis or CVS), and type of cytogenetic result (normal vs. abnormal) as covariates, showed that the only parameter

TABLE 2

Cytogenetic abnormalities diagnosed in singleton pregnancies achieved by ICSI after CVS amniocentesis.

Group	Abnormality	Amniocentesis (n)	CVS (n)	TOP
Inherited				
	45, XY, der (13;14)(q10;q10)	1	3	—
	45, XX, der (13;14)(q10;q10)	1	2	—
	45, XX, der (14;15)	1	—	—
	45, XX, der (14;21)(q10;q10)	—	1	—
	46, XY, der (14;21)(q10;q10), + 21	—	1	+
	46, XX, inv (10)(q11q23.2)	1	—	—
	46, XX, inv (1qh)	1	—	—
	46, XX, inv (9qh)	1	—	—
	46, XY, inv (1)	1	—	—
	46, XY, inv (5)	1	—	—
	46, XX, t (1;3)(p32;q23)	1	—	—
	45, XY, t (13q14q)/44,x,t (13q14q)	1	—	—
De novo				
	47, XX, + 21	—	1	+
	47, XY, + 21	2	—	++
	47, XX, + 18	—	1	+
	46, XY/47, XY,+21/48,XY,+21,+mar	—	1	+
	47, XXX	1	1	—
	47, XYY	1	1	+
	46, XY, inv (4)(p12;p16.2)	1	—	—
	46, XX, inv (9qh)	1	—	—
	46, XX/47, XXX	—	1	—
	45, X/46, XX/47, XXX (10,87,3)	—	1	+
	46, XX/46X, i (X)(q??))/47, XXx	1	—	—
	46, XX, t (2;13)	1	—	—
	46, XX, t (2;5)	1	—	—
Karyotype not available in both parents	46, XY, inv (7)(q22;q34)	1	—	—

TOP = termination of pregnancy. + = termination performed.

Kolibianakis. ICSI outcome after CVS and amniocentesis. Fertil Steril 2003.

exerting a significant ($P=.02$) influence on fetal loss was the type of sampling procedure (exp(B) = 4.4; 95% CI, 1.3–14.9).

Preterm Delivery and Birth Weight After Prenatal Diagnosis

A similar preterm delivery rate was observed between amniocentesis and CVS (11.2% vs. 12.4%, respectively). Moreover, no significant difference between the two methods was present in the proportion of babies with birth weight that was either <1,500 g (1.8% vs. 3.8%, respectively) or between 1,500 g and 2,500 g (8.2% vs. 4.6%, respectively). Comparison between amniocentesis and CVS regarding the chance of preterm delivery resulted in an odds ratio of 0.9 (95% CI: 0.5–1.7). In addition, considering the chance of delivering a baby with very low birth weight and low birth weight, the odds ratios (95% CI) were 0.4 (0.2–1.2) and 1.8 (0.7–3.9), respectively.

DISCUSSION

This study suggests that amniocentesis in singleton pregnancies achieved by ICSI is associated with a lower risk for fetal loss as compared with CVS. On the other hand, the type of prenatal procedure does not seem to result in a significantly different chance of preterm delivery or of birth of a baby with low or very low birth weight.

A prerequisite for reliable comparison of the risk of fetal loss between different prenatal genetic methods is their performance by the same experienced operators, as was the case in the current study. However, such a comparison can be confounded by several additional factors.

For instance, it is not possible to identify accurately whether a certain fetal loss is related or not to the procedure performed. This is because the time period during which the prenatal diagnostic procedure influences pregnancy outcome is not known with certainty. Therefore, all spontaneous fetal losses occurring after prenatal diagnosis should be included in the comparison of the two methods. Any additional factor that potentially contributes to fetal demise can be assumed to exist in both groups.

On the other hand, CVS and amniocentesis are not performed at the same gestational age. Consequently, an increased background risk for miscarriage exists in the CVS

group compared with the amniocentesis group. Moreover, the time available for pregnancy surveillance and therefore the chance for observing an adverse event is higher after CVS as compared with amniocentesis.

Efforts to overcome these problems in randomized comparison designs include the initiation of the observation period at the same gestational age, before prenatal testing is carried out. However, even in this situation, the period that the influence of the prenatal procedure per se is present is still higher after CVS.

In the current study, the observation period for amniocentesis and CVS started at the time of performing either method. Evidently, such a study design may introduce bias in favor of amniocentesis in calculating fetal loss risk. This is because miscarriages recorded after CVS and before the time that amniocentesis is performed are recognized as fetal losses. However, some of them would have occurred even without the presumably adverse influence of a prenatal genetic testing. On the contrary, miscarriages occurring at the same time period in the amniocentesis group remain unnoticed.

Nevertheless, in the present study, no fetal losses occurred in the CVS group before 16 weeks, and thus such a potential bias is avoided. This is probably a consequence of a significantly higher proportion of terminations performed in the CVS group as compared with the amniocentesis group. In this way, potential miscarriages in CVS patients before amniocentesis was performed were avoided, and thus a bias in favor of amniocentesis is probably minimal.

Termination of pregnancy was not considered to be fetal loss because this would have been an additional source of bias in favor of amniocentesis. Furthermore, the inclusion in the study of patients who underwent a second prenatal procedure would have confounded the comparison between the two methods, as a second procedure is not always of the same type as the initial one.

To the best of our knowledge, this is the first study comparing pregnancy outcome after CVS and amniocentesis in singleton ICSI pregnancies. Analogous comparisons between the two methods have been performed in natural conceptions in both randomized (9–11) and prospective designs (12).

Although a higher fetal loss rate was observed after CVS as compared with amniocentesis in the Canadian trial (9) as well by Rhoads et al. (12), the differences reported between the two methods (0.6% and 0.8%, respectively) did not reach statistical significance. On the contrary, the European randomized trial (10) suggested that CVS reduces the chances of a successful pregnancy outcome by 4.6% in comparison with second-trimester amniocentesis (95% CI: 1.6–7.5). Similarly, in a randomized comparison between the two methods (11), a significantly higher fetal loss rate was present in the combined (transvaginal and transabdominal) CVS group as compared with amniocentesis in a low–genetic risk population (difference: 2.16%). A meta-analysis (13) of the three randomized trials that currently exist (9–11) showed that CVS significantly increases the chance for fetal loss as compared with amniocentesis (odds ratio, 1.33; 95% CI, 1.17 to 1.52). Therefore, the existing literature and the present study support consistently a higher fetal loss rate after CVS than after amniocentesis.

Moreover, in agreement with the results of this study, no difference in terms of prematurity (10, 12) or birth weight (9) has been reported to occur between pregnancies in which either amniocentesis or CVS were performed. On the other hand, a higher proportion of cytogenetic abnormalities detected after CVS in comparison with amniocentesis has been observed in IVF patients (14). This has been attributed to the fact that pregnancies sampled by CVS could represent gestations that would have aborted before amniocentesis at 16 weeks.

The risk of fetal loss after different prenatal genetic procedures is probably the most important information that couples consider before deciding on the method of diagnosis. This is especially true in the case of ICSI pregnancies that both are valuable and carry an increased genetic background risk. The current study suggests that amniocentesis may result in a lower risk of fetal loss compared with the case of CVS in patients with singleton pregnancies achieved by ICSI. However, definite conclusions for this high-risk population on the absolute reduction of fetal loss should be drawn from a randomized, controlled trial.

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