Genetic sonography as the preferred option of prenatal diagnosis in patients with pregnancies following intracytoplasmic sperm injection

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The option of prenatal diagnosis with nuchal translucency measurement at 10–14 weeks of gestation and second trimester targeted ultrasound including fetal echocardiography (genetic sonography) is reported in patients after intracytoplasmic sperm injection (ICSI). From January 1995 to December 1998, 153 consecutive patients, with a mean age of 32.3 years (± 4.1) and 29.6% ≥ 35 years, who had become pregnant after ICSI, were studied. They attended our unit for first and second trimester sonography. Of these, 67.8% of primigravid and 80.9% of nulliparous women were included. Multiple pregnancy rate was 19.7%; 189 fetuses were screened in total. Due to the introduction of genetic sonography in 1995, the rate of invasive prenatal diagnosis decreased from 74% in 1995, to 48, 36 and 19% in 1996, 1997, and 1998 respectively. Two inherited numerical and structural chromosomal anomalies in clinically healthy children at birth (1.0%) and four major malformations in all liveborn children and late abortions (2.1%) were recorded. The results demonstrate that especially in women of advanced reproductive age with a long history of infertility, detailed genetic sonography may be a reasonable and highly accepted alternative to avoid even the relatively low risks associated with invasive screening procedures.

Key words: assisted reproduction techniques/chromosomal aberration/genetic sonography/intracytoplasmic sperm injection/targeted ultrasound

Introduction

Women with longstanding infertility who conceive after assisted reproduction techniques are usually older than pregnant women in the general population. Advanced maternal age (≥35 years) occurs in 25–46% (Doyle et al., 1992; Wennerholm et al., 1996) of pregnancies following in-vitro fertilization (IVF). The correlation between the increasing risk of chromosomal disorders with maternal age is well documented (Snijders et al., 1995).

Intracytoplasmic sperm injection (ICSI) is a successful therapeutic option for couples with male factor infertility (van Steirteghem et al., 1993; Palermo et al., 1996a). Prospective follow-up studies of the children from ICSI pregnancies demonstrated malformation rates within the expected range for the general population of 2.6–2.9% (Palermo et al., 1996b; Bonduelle et al., 1998a).

The possible genetic risks of this procedure and the occurrence of chromosomal abnormalities are thought to be major concerns. In total, 1082 prenatal karyotypes were performed in pregnancies after ICSI at the Centre for Reproductive Medicine at the Brussels Free University Hospital. In all, 1.66% de-novo chromosomal aberrations have been observed; half of these (0.83%) were sex chromosome anomalies and the other half autosomal aberrations (Bonduelle et al., 1998b). These data show that the prevalence of sex chromosome anomalies is slightly increased in ICSI fetuses when compared to unselected naturally conceived children. The higher incidence of inherited structural chromosomal aberrations among the children is predictable when partners with planned ICSI treatment have been cytogenetically investigated. Chromosomal disorders are diagnosed in 1.1–5.5% of infertile females and 2.1–4.5% of males with reproductive problems (Testart et al., 1996; Meschede et al., 1998a; Scholtes et al., 1998).

Recently, a prospective, controlled study of children born after ICSI (n = 89), IVF (n = 84) or natural conception (n = 80) at 1 year of age was published (Bowen et al., 1998). They found a significant delay in the mental development of boys born after ICSI compared to both control groups. On the other hand, a study was published on 201 children born after ICSI and 131 after IVF at 2 years of age which compared the mental development with a group of 1283 Dutch representative children after spontaneous conception (Bonduelle et al., 1998c). They could not find any abnormality or developmental delay, either in the ICSI or in the IVF group. One problem of the data of Bowen (Bowen et al., 1998) may be that there were frank differences between the social and economic status of the parents in the different cohorts, showing great disadvantages for the fathers and mothers of the children born after ICSI. Therefore, at the moment, the further postnatal development of children born after ICSI seems to be normal, but should be included in larger prospective studies.

Non-directive counselling of couples who undergo IVF/ICSI should be available for detailed evaluation of the patient’s family history and a possible underlying genetic cause of infertility, to counsel for advanced maternal age and to address the genetic risk of the procedure in terms of sex-chromosomal anomalies (Lawler and Gearhart, 1998). On the basis of the available data, patients pregnant through ICSI should have the choice of appropriate prenatal diagnosis.

Despite its widespread use in the international literature, the
term genetic sonography is inadequate and might be misleading. Especially when dealing with patients, it has to be pointed out that the so-called second trimester genetic sonography (de Vore and Alfi, 1995; Vintzileos et al., 1997) consists of a sonographic screening for markers of chromosomal abnormalities such as trisomy 21, 18 and 13. As some fetuses do not present such phenotypic markers, chromosomal abnormalities and the vast majority of genetic disorders can never be completely excluded by means of ultrasound. When looking for most monogenetic disorders, the only way to diagnose such defects accurately is through special microgenetic investigations. Patients therefore have to be counselled that this type of examination only enables a more individual risk calculation than the maternal age related risk but never gives a genetic diagnosis.

The nuchal translucency (NT) screening at 10–14 weeks of gestation allows the identification of fetuses with a high risk for chromosomal aberrations, structural abnormalities or genetic syndromes (Souka and Nicolaides, 1997; Snijders et al., 1998) as well as a high proportion of fetuses with major defects of the heart and great arteries (Hyett et al., 1999). In combination with maternal age, this method is an effective screening instrument for trisomy 13, 18 and 21 with a sensitivity of ~69–80% and a low false-positive rate (Snijders et al., 1996, 1998; Taipale et al., 1997). A small NT thickness can reduce the background risk based on maternal age alone. Given this information on their individual risk, patients are enabled to decide whether to undergo invasive prenatal testing by chorionic villous sampling (CVS) or amniocentesis (AC) with a small but significant procedure related risk of miscarriage.

At our institution, in a second step the genetic sonography is followed by a 16–22 week scan for ultrasonographic chromosomal markers and structural defects including fetal echocardiography. This is particularly important in patients with increased NT despite a normal karyotype, since the prevalence of cardiac defects and structural abnormalities increases with NT thickness (Souka et al., 1998). If the 16–22 week scan demonstrates a normally developed and grown fetus without any signs of chromosomal disorders or major defects, the individual risk can be reduced again. A recent review (Vintzileos et al., 1998) reported an overall sensitivity of second trimester high resolution ultrasonography for the detection of Down’s syndrome of 81% and a false-positive rate of 12%. In the case of a diagnosed fetal abnormality, karyotyping may be recommended to exclude a chromosomal disorder.

Materials and methods

From January 1995 to December 1998, 153 patients carrying 189 fetuses, all pregnancies established after IVF/ICSI treatment, were referred for first and second trimester sonography. Patients were divided into two groups.

Group I consisted of patients attending our unit for NT screening (n = 87 fetuses) at 10–14 weeks of gestation, which had an individual risk calculation based on maternal age and the measurement of the NT thickness. Measurements were taken according to the following criteria (Snijders et al., 1998): (i) obtaining a good sagittal section of the fetus, (ii) fetus occupies at least 75% of the image, (iii) placement of the callipers at the inner lines between the skin and the soft tissue overlying the cervical spine, and (iv) measurement of the maximum thickness.

An NT thickness of ≥3 mm was taken as a cut-off level (Taipale et al., 1997). All sonograms were performed by physicians experienced in first trimester scanning. According to their risk calculation patients had to decide to either undergo invasive prenatal testing and/or to continue with a 20 week scan. Most of the patients were counselled prior to the examination by a medical geneticist.

The patients of group II (n = 102 fetuses) were referred for a second trimester sonogram (16–22 weeks). In this group, 32 patients had opted for an elective amniocentesis in case of normal ultrasound examination, including two couples with diagnosed male aberrations. Standard fetal biometry with scoring of the amount of amniotic fluid and grading of the placenta as well as umbilical artery and uterine artery Doppler measurements were performed routinely. In addition, fetuses were examined by targeted ultrasound for structural defects and chromosomal markers (short femur, short humerus, pylectasis, increased nuchal fold thickening, echogenic bowel, choroid plexus cyst) followed by fetal echocardiography with colour Doppler flow mapping. If the scan was normal, patients were counselled and advised that the theoretical risk of having a child with a chromosomal disorder had been reduced again by 50–60% (DeVore and Alfi, 1995; Vintzileos et al., 1997). If one or more ultrasound markers or a major abnormality were detected, patients were counselled regarding the increased risk of a chromosomal anomaly and were offered amniocentesis or fetal blood sampling to determine the karyotype.

Information about the chromosomal analyses and pregnancy outcomes was obtained by reviewing the hospital charts, birth protocols and contacting the geneticists and paediatricians.

Results

In all, 122 singletons (80.3%), 23 twin (14.5%) and eight triplet pregnancies (5.2%) were included. The study group consisted of 67.8% primigravidae and 80.9% nulliparae. The mean maternal age was 32.3 years (range 24–42 years); 29.6% of the patients were ≥35 years and 43.4% were between 30 and 34 years old. Patients were referred for detailed ultrasonographic evaluation as well as for karyotyping. Further indications for prenatal diagnosis included advanced maternal age in 45 patients and abnormal karyotypes in two male partners: (i) 46,XY ps dic(15)(pter→q12;q12→pter). Angelman/Prader Willi region tested negative by fluorescent in-situ hybridization; (ii) 46,X,t(Y:22)(q11;q12).

We scanned 87 fetuses at 10–14 weeks of gestation including NT (NT) measurement (Figure 1). Nuchal translucency measurement was possible in all fetuses. The rate of NT screening increased from 28.2% in 1995 to 35.5% in 1996, to 54.7% in 1997 and 68.9% in 1998 (Table I). From the 87 fetuses, 81 had a normal NT thickness and in six cases the measured NT was ≥3 mm (1×4.7 mm, 1×3.5 mm, 2×3.4 mm, 2×3.1 mm). Chromosomal analysis was performed in all six cases and electively in 14 more fetuses by patient’s request, either by chorionic villus sampling or amniocentesis, all resulting in normal karyotypes. The ‘genetic sonogram’ was completed at 18–22 weeks of gestation and there were two cases of mild pylectasis in fetuses without first trimester karyotyping, but none of the 67 patients who had initially declined an invasive testing opted for karyotyping after the second screening.
A total of 102 fetuses was evaluated by second trimester sonography, the majority of them 88/102 (86.3%) had a normal scan with no ultrasound markers or structural defects present (Figure 2). In this group, 32 patients opted for an elective amniocentesis after the ultrasound screening. There were two inherited numerical chromosomal abnormalities diagnosed. The first case was a fetus with the karyotype 46,XX psu dic(15)(pter→q12;q12→pter) of a 37 year old woman and a partner with the same aberration and oligoasthenoteratozoospermia (OAT). In the second case, the father was carrier of a balanced translocation [46,t(y;22) (q11;q12)]. His 28 year old wife achieved a twin pregnancy. One fetus presented a normal 46, XX karyotype, the other had an unbalanced translocation [45,X,del(Y;22)(q11;q12)]. The fetus was partially monosomic for chromosome 22. The parents refused further investigations for Di George syndrome. The ultrasound appearance of both fetuses with chromosomal aberrations was completely normal and the children were clinically healthy at birth.

Abnormal second trimester ultrasound findings were present in 14 fetuses, including three fetuses from twin pregnancies. There were six cases (one twin) of mild pyelectasis (anteroposterior diameter of the renal pelvis of ≥5 mm), one massive hydronephrosis, one case of bilateral choroid plexus cysts of 3 mm and 3.6 mm, one duodenal obstruction (one twin), one duodenal atresia with an abnormal vascular course, one fetus with omphalocele, one fetus with spina bifida (one twin) and two cases of early intrauterine growth retardation (IUGR) with highly abnormal Doppler parameters in the umbilical and uterine arteries. Both fetuses with IUGR died before the 19th week of gestation. Autopsy confirmed the diagnosis and excluded malformations or abnormal karyotype. With the exception of the two fetuses with massive hydrencephrosis and with duodenal atresia respectively, the other 12 fetuses with abnormalities as well as the two fetuses from twin pregnancies, where only one fetus had an abnormal scan, were karyotyped, giving normal chromosomes in all cases. In a twin pregnancy where one fetus was affected with spina bifida, amniocentesis of both fetuses at 20 weeks gestational age was performed. The patient developed a severe cervical insufficiency at 22 weeks. An emergency cerclage was not successful and premature rupture of membranes occurred resulting in late abortion.

The children were followed up postnatally and the antenatal diagnosis confirmed. No further anomalies were recorded. A malformation rate of 2.1% (4/189) was noted if spina bifida, duodenal atresia, duodenal obstruction and omphalocele were classified as major malformations.

The total amount of invasive prenatal diagnosis in both groups decreased from 73% in 1995, when patients were advised to have karyotyping, to 62% in 1996, 36% in 1997 and 19% in 1998, which means a reduction of 54% (Table I). The decrease is mainly the result of the reduction of the total number of genetic amniocenteses because of the ICSI treatment. Comparing the two groups, there are ~15% fewer invasive procedures in group I (NT-screening + second trimester sonography) which represents fewer elective chromosome analyses (Figures 1 and 2). Mean maternal age distribution was comparable in both groups.

### Table I. Rate of nuchal translucency (NT) screening and karyotyping in 189 fetuses after IVF/ICSI per year

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of fetuses</th>
<th>Mean maternal age (years)</th>
<th>Rate of NT screening (%)</th>
<th>Rate of karyotyping (%)</th>
<th>Age ≥35 years without karyotyping (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>21</td>
<td>32.1 ± 3.9</td>
<td>28.2</td>
<td>73.4</td>
<td>6.7 (1/15)</td>
</tr>
<tr>
<td>1996</td>
<td>30</td>
<td>32.1 ± 3.9</td>
<td>35.5</td>
<td>62.5</td>
<td>8.3 (2/24)</td>
</tr>
<tr>
<td>1997</td>
<td>64</td>
<td>32.6 ± 4.1</td>
<td>54.7</td>
<td>36.0</td>
<td>16.1 (9/56)</td>
</tr>
<tr>
<td>1998</td>
<td>74</td>
<td>32.2 ± 4.3</td>
<td>68.9</td>
<td>18.9</td>
<td>19.0 (11/58)</td>
</tr>
<tr>
<td>1995–1998</td>
<td>189</td>
<td>32.3 ± 4.1</td>
<td>46.5</td>
<td>35.3</td>
<td>15.0 (23/153)</td>
</tr>
</tbody>
</table>
Discussion

In the general population the percentage of women >35 years delivering is ~8–15% (Pandya et al., 1995a); the perinatal statistics for our region in Schleswig-Holstein gives 15% in 1997. These figures are much higher in women with longstanding infertility. In the study group, 29.6% of the patients were of advanced maternal age, with a high percentage of primigravidae and nulliparae. The positive correlation between maternal age and the risk of autosomal chromosomal disorders is well documented, this group contributes to ~30% of cases of trisomy 21 (Snijders et al., 1995). The traditional way of identifying these fetuses is to offer invasive prenatal testing. However, considering the long history of infertility and even if the procedure-related risk for miscarriage is low, this option might be not acceptable for some couples. This is still true, even if others have recently shown that there is no increased risk of preterm deliveries, low birth weight, very low birth weight or increased fetal loss rate following either amniocentesis or chorionic villus sampling in pregnancies established after an ICSI procedure (Aytoz et al., 1998).

We recommend an individual risk calculation and genetic counselling for each patient. On the basis of maternal age, the week of pregnancy and the history of former pregnancies with chromosomal disorders the background risk is defined. In combination with NT screening at 10–14 weeks of gestation as an effective screening method for fetuses with increased risk of chromosomal defects (Snijders et al., 1998), each patient’s risk can be calculated. We used a cut-off level of ≥3 mm as previously described with a sensitivity of 69% for all aneuploidies and a specificity of 99% with a false positive rate of 0.8% (Taipale et al., 1997). In another study of 1273 women, using a cut-off level of 2.5 mm, an NT measurement of <2.5 mm was associated with a 4.5-fold reduction of the maternal-age related risk for fetal trisomies, whereas an NT >2.5 mm was associated with a 12-fold increasing risk (Nicolaides et al., 1994).

The risk for chromosomal defects rises with NT thickness; this correlation was found to be positive for trisomy 21, trisomy 18, trisomy 13, Turner syndrome and triploidy (Pandya et al., 1995b). Because of the normal increase of NT thickness with gestation, the King’s College group calculated the risk on the basis of CRL, maternal age and NT measurement (Snijders et al., 1996). A combination of these results with other biochemical markers is possible. Recently, a screening programme for trisomy 21 at 10–14 weeks was introduced using fetal NT, maternal serum free beta-human chorionic gonadotrophin and pregnancy–associated plasma protein-A. The detection rate was 89% at a fixed false-positive rate of 5% (Spencer et al., 1999). Another new approach to non-invasive prenatal diagnosis is the combination of fetal NT screening with maternal age and enrichment of fetal cells from maternal blood. Preliminary results suggest that the sensitivity for trisomy 21 remains at ~80%. For the future however this could reduce the need of fetal karyotyping in high-risk patients from 5% to <1% (Al-Mufti et al., 1998). In contrast, the biochemical screening and the enrichment of fetal cells have restricted application in multifetal pregnancies, which are frequent after IVF/ICSI. NT screening for fetal trisomy 21, however, had a similar sensitivity in twins when compared to singleton pregnancies (Sebire et al., 1996).

In a continuing study of 61 972 singleton pregnancies, 20 cases of 47,XXY; 47,XY or 47,XXX were identified. In 40% the NT was above the 95th centile. But compared with an expected number of 104 cases of these sex chromosomal anomalies in this population, only 9% of potential livebirths would be positive by NT measurement and maternal age (Sebire et al., 1998). Therefore, it is difficult to assess if there is an increased NT in those fetuses suffering from sex aneuploidies. Most probably, the sex chromosome aberrations which are reported in the series of Bonduelle et al. (Bonduelle et al., 1998b) would not have been diagnosed with NT screening and genetic sonography alone. However, with the exception of Turner syndrome, major congenital malformations are not present in those children, and mental retardation does not occur more often than in a control population, although academic achievement may be reduced (Meschede and Horst, 1997). Others have reported that only a small proportion of parents (12.7%) would choose elective abortion of those pregnancies, which are complicated by a sex chromosome aberration (Meschede et al., 1998b).

This fact is important, since 1.66% de-novo chromosomal aberrations in a series of 1082 prenatal tests in pregnancies established after an ICSI treatment have been observed (Bonduelle et al., 1998b). In all, 0.83% of those were aberrations of sex chromosomes, which increase towards the expected rate of 0.19–0.23% in an unselected population. However, in absolute numbers, there were only nine out of 1082 prenatal tests with de-novo sex-chromosomal aberrations, which is still a very low number. Therefore, the only conclusion from that observation should be to counsel those couples undergoing an ICSI treatment that there might be a slight increase in sex chromosome aberrations. More results have to be collected to confirm this observation. In particular, the incidence of sex chromosomal aberrations in spermatozoa from men suffering from oligozoospermia has to be further evaluated. Some authors described an increased rate of chromosomally abnormal spermatozoa (Moosani et al. 1995) but others did not (Gutenbach et al., 1997). It is important to know that very high numbers of spermatozoa (~100 000) must be studied with several fluorescent probes for different chromosomes, before this question can be reliably and statistically answered correctly. In our study, two cases of paternally inherited chromosomal aberrations have been recorded. The amniocentesis in these cases was performed electively, since the ultrasound examination was completely normal. The children were phenotypically normal at birth and at paediatric follow-up.

The malformation rate in our series of 2.1% is comparable to the 2.9% described elsewhere (Bonduelle et al., 1998a) in 1987 liveborn children, stillbirths and late abortions. It is important to note that all studies, which have reported on malformations after ICSI, have found a normal malformation rate (Palermo et al., 1996b; Wennerholm et al., 1996; Bonduelle et al., 1998a,c; Bowen et al., 1998; Govaerts et al., 1998; Loft et al., 1998; Ludwig et al., 1999).
To conclude, from our experience it is useful to offer couples who have become pregnant after ICSI, a two-step genetic sonography, which allows a more individualized risk calculation. The couples undergoing ICSI should be counselled that there might be a slight increase in sex chromosome aberrations (Johnson, 1998). The medical consequences of those aberrations have to be explained to the parents. In our series an increasing proportion of women with pregnancies conceived after ICSI preferred genetic sonography in the first and second trimester. However, it has to be guaranteed that this sonography is performed by an experienced sonographer, using high resolution ultrasound. It has previously been reported that 82% of patients, who had become pregnant after ICSI strongly favoured non-invasive prenatal testing, if they had the option (Meschede et al., 1998c). This can be confirmed by our data. In general, there is an increasing trend to use detailed ultrasound evaluation. Abnormal scan results are the most important factor in women’s decisions to undergo invasive procedures for chromosome analysis (Vintzileos et al., 1997). The strategy of counselling ICSI patients about the concept of genetic sonography and avoiding invasive prenatal testing after successful treatment was found to be highly acceptable in our patients.

References
A. Geipel et al.


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