

INCREASED INCIDENCE OF CYTOGENETIC ABNORMALITIES IN CHORIONIC VILLUS SAMPLES FROM PREGNANCIES ESTABLISHED BY *IN VITRO* FERTILIZATION AND EMBRYO TRANSFER (IVF-ET)

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SUMMARY

We studied 201 pregnancies that were established by *in vitro* fertilization and embryo transfer (IVF-ET) and compared the frequency of cytogenetic abnormalities with that found in a large control population matched for indication group (advanced maternal age) and time of sampling. A total of 252 IVF-ET fetuses were cytogenetically analysed by either chorionic villus sampling (CVS; $n=80$) or amniocentesis ($n=172$). Eleven chromosome abnormalities were found in the CVS group (13.8 per cent); among them, a 45,X/46,X,dic(Y)(q11)/46,X,del(Y)(q11) mosaic that was found in an IVF pregnancy established by intracytoplasmic sperm injection (ICSI), four cases of trisomy 21, and three cases of trisomy 7 confined to the placenta. The results indicate a statistically significant three- to five-fold increase in both confined placental abnormalities ($P<0.008$) and true fetal chromosome anomalies ($P<0.04$). In the amniocentesis group, identical rates (1.7 per cent) of chromosome abnormalities were found in the IVF-ET and control groups. It is concluded that late first trimester, but not early second trimester, IVF-ET pregnancies are characterized by an increased frequency of cytogenetic abnormalities found at prenatal diagnosis.

KEY WORDS: chorionic villi; amniocentesis; mosaicism; IVF-ET; ICSI

INTRODUCTION

The frequency of cytogenetic abnormalities in fetuses and neonates from pregnancies established by *in vitro* fertilization and embryo transfer (IVF-ET) has been the subject of a number of reports documenting birth defects in this population (AIHW, 1993; Rizk *et al.*, 1991; Testart *et al.*, 1992). The frequency of unbalanced chromosome aberrations was found to be comparable to that

found in the general population and an IVF origin of pregnancy is generally not considered to be sufficient indication for invasive prenatal diagnosis. However, IVF pregnancies are characterized by a high frequency of spontaneous abortions between 6 and 20 weeks of gestation (AIHW, 1993), possibly indicating an increased frequency of cytogenetic abnormalities in the early IVF pregnancy, while very few studies are available that document prenatal findings in IVF populations (Würfel *et al.*, 1992). We therefore performed a retrospective study to establish the incidence of cytogenetic abnormalities in IVF pregnancies of women referred for prenatal diagnosis because of advanced maternal age.

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MATERIALS AND METHODS

Between January 1990 and August 1994, a total of 201 women whose pregnancies were established by *in vitro* fertilization and embryo transfer (IVF-ET) at various centres in The Netherlands and Belgium were referred to our hospital for prenatal diagnosis because of advanced maternal age (≥ 36 years at 18 weeks of pregnancy). One pregnancy was established by intracytoplasmic sperm injection (ICSI) (Lanzendorf *et al.*, 1988; Palermo *et al.*, 1992) because of azoospermia; all the others were established by standard IVF procedures. The maternal age indication for prenatal diagnosis in the case of a donor oocyte was based on both the patient's and the donor's age. After appropriate counselling, 62 women opted for transabdominal chorionic villus sampling (TA-CVS) and 139 women opted for amniocentesis.

TA-CVS was performed at 11–14 weeks of gestation and 5–20 mg of villi was obtained under ultrasound guidance (Jahoda *et al.*, 1990). Villi were incubated overnight using fluorodeoxyuridine (FdU) synchronization (Gibas *et al.*, 1987). A total of 80 karyotypes were obtained from 46 single, 14 twin, and 2 triplet pregnancies.

Amniocentesis was performed at week 16–18 of gestation and 20 ml of amniotic fluid was aspirated under ultrasound guidance. Amniocytes were cultured in Chang medium on glass coverslips; *in situ* preparations were karyotyped after trypsin–Giemsa staining. A total of 172 karyotypes were obtained from 110 single, 25 twin, and 4 triplet pregnancies.

The frequency of cytogenetic abnormalities in the IVF-ET population was compared with that in the control population consisting of pregnancies that were not established by IVF-ET and referred for prenatal diagnosis to our centre during the same period because of advanced maternal age. Control populations consisted of 4406 CVS and 3251 amniocenteses. Statistical analysis, in which the frequency of cytogenetic abnormalities in the various groups was compared for each age category, was carried out by the exact Mantel–Haenszel procedure. Odds ratios were corrected for both maternal age and gestational age in the CVS group.

Fluorescent *in situ* hybridization (FISH) was performed on cultured amniocytes as described previously (Van Opstal *et al.*, 1993). The following probes were applied: pDP105 (DYZ4) (Gal *et al.*, 1987) hybridizing to Yp; pDP97 (DYZ3) (Wolfe

et al., 1985) hybridizing to the Y centromere; p49f (DY31) (Ngo *et al.*, 1986) hybridizing to Yq11; and AmprobeRPN1305X (Lau, 1985) hybridizing to the heterochromatic part of Yq.

RESULTS

In chorionic villus biopsies from IVF-ET pregnancies, a total of 11 cytogenetic abnormalities were found (excluding single cell aneuploidy and familial structural abnormalities), consisting of six fetal abnormalities and five confined placental mosaics (Table I). The fetal abnormalities comprised a case of XYY and four cases of aneuploidy for chromosome 21; all four cases were accompanied by echographic abnormalities (hydrops fetalis and/or hygroma colli) that were detected at the time of biopsy at 11–14 weeks of gestation. Two of these cases involved twin pregnancies which were selectively terminated. No material for confirmation of the fetal karyotype could be obtained from the selectively terminated cases. The single pregnancy that was established by intracytoplasmic sperm injection (ICSI) presented with a mosaic 45,X/46,X,dic(Y)(q11)/46,X,del(Y)(q11) which was confirmed in amniotic fluid cells and fetal fibroblasts. Blood samples from both parents yielded normal karyotypes in 100 metaphases. The two derivative Y chromosomes (Fig. 1A) were characterized by FISH: both derivatives were negative with AmprobeRPN1305X and p49f indicating the absence of the heterochromatic region and substantial loss of euchromatin; probes pDP105 and pDP97 both showed two signals on the dic(Y)(q11) (Figs 1B and 1C) and one signal on the del(Y)(q11). The pregnancy was terminated at the parents' request; obduction showed a male fetus without apparent congenital malformations.

Comparison of the frequency of fetal cytogenetic abnormalities in IVF pregnancies sampled by CVS (6/80; 7.5 per cent) with that in a control population (75/4406; 1.7 per cent) indicated a significant increase in the frequency of cytogenetic abnormalities in the IVF group ($P < 0.04$; odds ratio corrected for both maternal and gestational age 3.6; 95 per cent confidence interval 1.1–9.6).

The five cases of confined placental mosaicism comprised one case in which both an additional chromosome 7 and an additional chromosome 13 were present, two cases with trisomy 7, and two cases of sex chromosome mosaicism. In the control population a total of eight cases involving an

Table I—Cytogenetic abnormalities in chorionic villus samples from IVF-ET patients referred for advanced maternal age

Case No.	Age (years)	Gestational age (weeks)	Karyotype	Follow-up
<i>Fetal abnormalities</i>				
1*	37	13	47,XY,+21	Selective TOP; NF
2*	36	12	47,XY	Continued pregnancy
3*	38	12	47,XX,+21	Selective TOP; NF
4	43	14	47,XY,+21	TOP; 47,XY,+21 (F)
5	39	12	46,XX/47,XX,+21 (2/28)	TOP; 47,XX,+21 (F)
6	38	13	45,X/46,X,dic(Y)(q11)/46,X,del(Y)(q11) (4/14/16)	45,X/46,X,dic(Y)(q11)/46,X,del(Y)(q11) TOP (AC; F)
<i>Placental abnormalities</i>				
7	39	12	45,X/46,XY (12/22)	NF; normal boy
8*	38	12	46,XY/47,XY,+7/48,XY,+7,+13 (8/2/40)	46,XY (AC)
9	37	11	46,XY/47,XY,+7 (11/21)	46,XY (LTC; AC)
10*	40	13	45,X/46,XX (2/18)	46,XX (AC)
11	35	11	47,XX,+7	46,XX (AC)

AC=Amniocentesis; LTC=long-term villus culture; F=fetal skin biopsy; TOP=termination of pregnancy; NF=no cytogenetic follow-up. An asterisk denotes a twin pregnancy; a normal karyotype was found by CVS in the second fetus.

additional chromosome 7 were present. Comparison of the frequency of all placental cytogenetic abnormalities (5/80; 6.2 per cent) with that in a control population (55/4406; 1.2 per cent) indicated a significant five-fold increase of placental cytogenetic abnormalities in the IVF population sampled by CVS ($P<0.008$; odds ratio corrected for both maternal and gestational age 5.6; 95 per cent confidence interval 1.6–15.2).

In amniotic fluid samples obtained from IVF-ET pregnancies, a total of three cytogenetic abnormalities were found (excluding pseudomosaicism and familial structural abnormalities). All three cases involved trisomy 21 (Table II). The frequency of non-familial cytogenetic abnormalities in the IVF population was 3/172 (1.7 per cent), which is identical (odds ratio corrected for maternal age 1.0; 95 per cent confidence interval 0.2–3.2) to the frequency in the control population of 56/3251 (1.7 per cent).

DISCUSSION

The present study documents cytogenetic abnormalities in a population of 252 fetuses from pregnancies of women referred for prenatal diagnosis because of advanced maternal age whose pregnancy was established by *in vitro* fertilization and

embryo transfer (IVF-ET). It compares the frequency of such abnormalities with that in a control population matched for indication group and period of sampling, and concludes that the frequency of cytogenetic abnormalities found in chorionic villi sampled at 11–14 weeks of gestation is significantly increased in the IVF-ET pregnancies. In contrast, the frequency of such abnormalities encountered after amniocentesis at 16–18 weeks of gestation was found to be similar to that in the controls.

Chromosome abnormalities found in chorionic villi can be divided into true fetal abnormalities and abnormalities confined to the placenta. A three- to four-fold higher frequency of fetal abnormalities was found in the IVF-ET population: apart from four cases of trisomy 21 and a case of XYY, a complex chromosome mosaic 45,X/46,X,dic(Y)(q11)/46,X,del(Y)(q11) was found in the single pregnancy that was established by intracytoplasmic sperm injection (ICSI). Combinations of XO and dicentric Yp or deleted Yq lines have been described previously, with patients presenting with a wide range of different phenotypes (Hsu, 1994). Apart from the present finding, a total of five other chromosome aberrations have been described after ICSI (Bonduelle *et al.*, 1994). Interestingly, all the cases involve sex chromosome abnormalities, possibly indicating that the ICSI

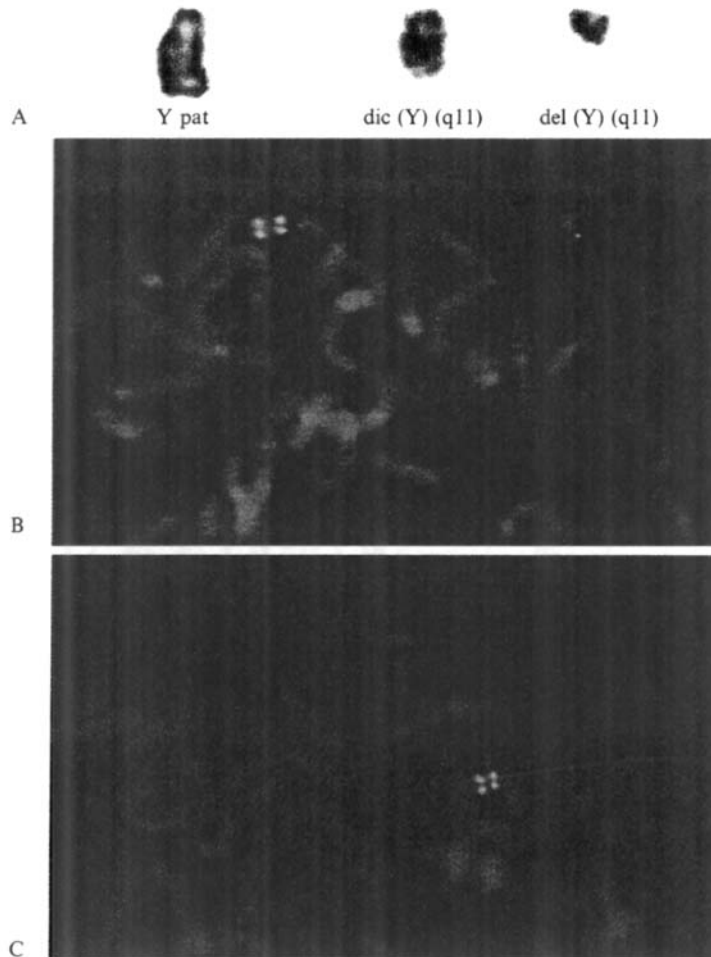


Fig. 1—(A) Paternal Y chromosome (left) and fetal derivative Y chromosomes (right) in a pregnancy established by intracytoplasmic sperm injection (ICSI) (GTG banding). (B) Partial metaphase with fluorescent *in situ* hybridization signals after hybridization with the Y centromere probe pDP97 and (C) the Yp probe pDP105 showing two signals on both chromatids of the dic(Y)(q11)

technique is associated with an increased prevalence of sex chromosomal aberrations. As ICSI bypasses sperm selection and involves the injection of an entire spermatozoon into the ooplasm, it can be speculated that the increase is either due to the ICSI technique itself or is related to the (paternal) fertility problems which have led to the choice of ICSI as fertility treatment.

The frequency of confined placental abnormalities was found to be five-fold higher in the IVF-ET population sampled by CVS. Apart from two cases with a low-grade mosaic 45,X, three cases of trisomy 7 were found; both types of

aneuploidy are a relatively common finding in CVS (Sachs *et al.*, 1990), but the frequency of trisomy 7, either alone or in conjunction with another trisomy, was found to be 20-fold higher in the IVF-ET group than in the controls. The finding of (mosaic) trisomy 7 in a chorionic villus biopsy has not been reported to be associated with the presence of (mosaic) trisomy 7 in the fetus, but may be associated with a higher risk of cystic fibrosis (CF) through uniparental isodisomy from a mutation carrier (Spence *et al.*, 1988). If an increased frequency of trisomy 7 placentae is not only present in classical IVF pregnancies, but is

Table II—Cytogenetic abnormalities in amniotic fluid samples from IVF-ET patients referred for advanced maternal age

Case No.	Age (years)	Gestational age (weeks)	Karyotype	Follow up
1*	41	16	47,XX,+21	47,XX,+21 (FB) Selective TOP
2	40	18	47,XY,+21	TOP; NF
3	39	16	46,XY/47,XY,+21 (3/28)	46,XY/47,XY,+21 (F) (6/40); TOP

FB=Fetal blood; F=fetal skin biopsy; NF=no cytogenetic follow-up; TOP=termination of pregnancy. An asterisk denotes a twin pregnancy; a normal karyotype was found after amniocentesis in the second fetus.

also present after intracytoplasmic sperm injection (ICSI), this finding may be important as ICSI treatment is considered the fertility treatment of choice in males with congenital bilateral absence of the vasa deferentia (Devroey *et al.*, 1994), a condition known to be associated with (compound) heterozygosity for mutations in the CF gene (Anguiano *et al.*, 1992).

In the IVF-ET group sampled by amniocentesis, the frequency of chromosome aberrations was not found to be different from that in a large control population; this contrasts with the finding in the IVF-ET group sampled by CVS in which marked differences with the controls were observed. Several factors may account for the different amniocentesis and CVS results: a high level (29 per cent) of spontaneous abortions was reported in the group of IVF pregnancies with a maternal age over 35 years (AIHW, 1993). The fetal cytogenetic abnormalities observed after CVS may thus represent pregnancies that would have aborted spontaneously before amniocentesis at 16 weeks of gestation. This possibility is supported by the observation that all four cases of trisomy 21 found in this group showed hydrops fetalis and/or hygroma colli, conditions that, if the pregnancy had not been terminated at the parents' request, might have led to a spontaneous abortion. Analysis of spontaneous abortions after IVF has documented chromosomally abnormal cases aborted between 11 and 16 weeks of gestation (Plachot, 1989). If an IVF pregnancy has an increased risk of a spontaneous abortion associated with a chromosome abnormality, it could be argued that prenatal diagnosis via amniocentesis at 16 weeks of gestation is the

most suitable approach in these pregnancies (excluding the multiple gestations) as it would avoid unnecessary interventions. On the other hand, the increased risk of a confined placental abnormality argues in favour of CVS in those cases in which uniparental disomy, especially of chromosome 7, could be of importance, as it is in pregnancies at risk for cystic fibrosis.

An alternative explanation for the difference between the amniocentesis and CVS groups may be found in the vanishing twin hypothesis (Tharapel *et al.*, 1989), in which resorption of a co-twin results in a cytogenetically mixed placenta containing cells from both the continuing pregnancy and the vanished twin. As IVF pregnancies are characterized by a high frequency of twin and triplet gestations, some of which spontaneously reduce to singleton and twin gestations, such a model may help to explain the increased frequency of placental anomalies found in CVS. However, in this model, discrepancies may be expected to occur between the sex established by CVS and that found after examination of fetal tissues; none has been found in the present series. The results of this retrospective study emphasize the continuing need for a close (cytogenetic) follow-up of pregnancies established by IVF-ET.

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