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Neonatal follow-up of 995 consecutively born children after embryo biopsy for PGD

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BACKGROUND: Outcome data on children born after assisted reproduction treatments are important for both patients and health-care providers. The objective of this study was to determine whether embryo biopsy as performed in PGD has an impact on the health of infants up to 2 months of age.

METHODS: A prospective comparative follow-up study of children born after PGD and children born after ICSI by collecting written reports and performing a physical examination at 2 months was performed. Auxological data at birth and physical findings up to 2 months of age were compared for 995 children consecutively live born after embryo biopsy (1994–2009) and for a control group of 1507 children born after ICSI with embryo transfer on Day 5.

RESULTS: No differences regarding mean term, prematurity (term <32 w and <37 w), mean birthweight, very low birthweight (<1500 g), perinatal death, major malformations and neonatal hospitalizations in singletons and multiples born following PGD versus ICSI were observed. Compared with ICSI, fewer multiples born following PGD presented a low birthweight (<2500 g) ($P = 0.005$).

CONCLUSIONS: Embryo biopsy for PGD does not introduce extra risk to the overall medical condition of newborn children. Multiples born following embryo biopsy appear to be at lower risk for low birthweight compared with multiples born following ICSI.

Key words: embryo biopsy / birthweight / ICSI / PGD / neonatal follow-up

Introduction

The specific aim of this study was to evaluate the possible effect of a one-or two-cell biopsy of a cleavage stage embryo obtained via IVF, on the health of live born children. An embryo biopsy is performed to allow PGD, first reported in the early 1990s, in order to determine structural and numerical chromosomal imbalances, specific monogenic defects and gender, as well as aneuploidy before embryo transfer to the uterus (Sermon *et al.*, 2004). ICSI, introduced in 1991 to treat male infertility with injection of one spermatozoon through the oocyte membrane, is a more sophisticated technique compared with classical IVF and bypasses natural sperm selection (Palermo *et al.*, 1992). In PGD, the use of ICSI is mandatory when the diagnosis is based on PCR to minimize the risk of contamination by residual sperm DNA. PGD is increasingly used for couples with a genetic risk combined with or without infertility, who want to avoid pregnancy interruption (Verlinsky *et al.*, 2004; Verpoest *et al.*, 2009).

Cleavage-stage biopsy of the embryo is accomplished by making a hole in the zona pellucida using either a stream of acidic Tyrode's solution or by laser, as previously described (De Vos and Van Steirteghem, 2001; Harton *et al.*, 2011). One or two aspirated blastomeres are collected in dedicated test tubes or fixed on glass slides prior to genetic testing. For monogenic disorders, the appropriate PCR-based assay is applied. In case of X-linked recessive disorders, sexing of the embryos by fluorescence in-situ hybridization (FISH) is offered if no specific PCR assay is available. For chromosomal aberrations, a specifically designed FISH procedure is used (Sermon *et al.*, 2004; Harper *et al.*, 2010).

So far, a limited number of reports on the medical outcome of children born after PGD have been published, but they are reassuring. No higher rates of congenital defects were observed at birth in children conceived by IVF/ICSI in association with PGD (Strom *et al.*, 2000; Goossens *et al.*, 2009; Liebaers *et al.*, 2010). The European Society of Human Reproduction and Embryology PGD Consortium reports

that characteristics at birth were comparable to those of ICSI babies (Bonduelle *et al*., 2002; Sermon *et al*., 2007).

Outcome data on children born after assisted reproduction treatments (ARTs) are important for patients and health-care providers. Therefore, a prospective study was set up to compare data on medical outcome of children born after PGD with children born after ICSI with embryo transfer on Day 5, conceived at our Centre between 1993 and December 2008. The control group of children born after ICSI with embryo transfer on Day 5, similar to the procedure after PGD, and during the same study period was included to determine whether potential differences in children's outcome could be exclusively attributed to the embryo biopsy.

Materials and Methods

Definitions

PGD refers to PGD for high genetic risk indications as well as to PGD-AS (PGD for aneuploidy screening). A stillbirth is an intra-uterine or intrapartum death of a child born at a gestation of ≥20 weeks and/or with a birthweight of ≥500 g. A neonatal death is defined as a demise of a live born within 7 days after birth. A perinatal death is either a stillbirth or a neonatal death. Prematurity was defined as birth before 37 weeks of gestation. The total malformation rate was defined as the sum of affected live births and stillborns for malformations divided by the sum of live births and stillbirths.

Study subjects

In this study 995, live born PGD children (670 singletons, 308 twins and 17 triplets) conceived between January 1994 and December 2008 are compared with 1507 live born ICSI children (1059 singletons, 433 twins and 15 triplets) conceived during the same time period. Future parents were recruited for this prospective clinical follow-up study before starting PGD or ICSI and enrolled following a written informed consent. Data on 581 of these children have been reported earlier (Liebaers *et al*., 2010).

Study procedure

Children of both study groups were examined at the age of 2 months at the Centre for Medical Genetics of the Vrije Universiteit Brussel (Brussels, Belgium) in accordance with a standardized protocol that included a medical history and physical examination by an experienced paediatrician who was blinded to the type of ART (Desmyttere *et al*., 2009). Biometrical data, such as weight, height and head circumference, were collected with standard equipment and according to a standardized procedure (www.vub.ac.be/groeicurven). Physical examination included a standardized assessment of major malformations. Major malformations were classified according to criteria previously defined (Bonduelle *et al*., 2002). A major malformation causes functional impairment and/or requires surgical correction (Bonduelle *et al*., 2002). Information about the ethnic origin, height and weight, as well as information about the maternal age at the birth of the child, parity, maternal health during pregnancy and history of alcohol and/or nicotine abuse during pregnancy were obtained by questionnaire.

Term, birthweight and admission to a neonatal care unit were obtained from medical files from hospitals and well-baby clinics ('Kind en Gezin' and 'Oeuvre Nationale des Enfants') with the consent of the parents.

Birthweight standard deviation scores (SDS) were calculated from the reference data of the Flemish growth survey in 2004 (Roelants *et al*., 2009).

Power and sample size calculations

Estimation of sample size was based on a 2.7% malformation rate in children born after natural conception (data from the national Belgian birth registry for 1989–2002 for all pregnancy interruptions, all stillborn (>20 weeks) and live born children until the age of 1 year), and considering a doubling in malformation rate among PGD children as clinically relevant. We calculated that a sample size of 1740 children total would be required to detect a doubling in major malformations for an alpha level of 0.05 and 80% power (PASS 11, NCSS, Kaysville, Utah, USA).

Statistical analysis

Data are presented as mean ± SD (continuous variables) and as number of cases or percentages including nominator and denominator values (categorical variables) for each group of interest. Statistical analysis for comparing PGD and ICSI groups included the Student's *t*-test (continuous variables) and the Fisher's exact test (categorical variables). A significance level of $P < 0.05$ was accepted throughout.

Multivariable (linear regression and logistic regression) analyses were conducted for each outcome of interest to explore the impact of PGD and ICSI simultaneously, adjusting for maternal age, pre-pregnancy BMI, parity, nicotine abuse, intake of alcohol and complications during pregnancy. Given the very small number of children with a major malformation, multivariable (logistic regression) analyses were not conducted for this outcome.

Ethics

The study was approved by the ethical committees of the University Hospital Brussels, and written informed consent was obtained from the parents.

Results

In the study group a total of 4300 IVF cycles with PGD were performed, leading to 1236 cycles with positive HCG values while in the control group 7246 ICSI cycles resulted in 3315 cycles with positive HCG values. Of the 1022 PGD children, 27 children were stillborn (2.6%) resulting in 995 children (670 singleton, 308 twin and 17 triplet children) born alive, of whom 9 (8 twin children and 1triplet child) died neonatally. Of the 1542 ICSI children in the control group, 35 children were stillborn (2.3%) resulting in 1507 children (1059 singleton, 433 twin and 15 triplet children) born alive of whom 10 (2 singleton and 8 twin children) died neonatally. Perinatal death rates among PGD versus ICSI singletons and PGD versus ICSI multiples did not differ (Table I). More specifically, there were eight perinatal deaths [1.2%; 95% confidence interval (CI) 0.6–2.3] among the PGD singletons and 21 perinatal deaths (1.9%; 95% CI 1.3–3.0) among the ICSI singletons [odds ratio (OR) 0.601; 95% CI 0.229–1.423; $P = 0.26$]. Among the PGD multiples, there were 28 perinatal deaths (8.1%; 95% CI 5.7–11.5) and 24 perinatal deaths (5.2%; 95% CI 3.5–7.6) among the ICSI multiples (OR 1.625; 95% CI 0.889–2.986; $P = 0.11$).

The mean birthweight for PGD singletons (3262.8 ± 543.5 g), PGD multiples (2299.8 ± 581.1 g) (twins: 2345.9 ± 552.0 g and triplets: 1394.1 ± 458.7 g) and the number of PGD neonates (10 singletons and 17 multiples) with a very low birth weight (<1500 g) was comparable with the ICSI babies. Significantly more ICSI multiples presented a low birthweight (<2500 g), more specifically 268 (17.8%) ICSI versus 161 (16.2%) PGD babies ($P = 0.005$). There were no differences in

Table I Perinatal deaths of children born following PGD compared with children born following ICSI[a].

n	PGD					Perinatal death rate (%)	ICSI					Perinatal death rate (%)	*P***
	LB	SB	ND	PD	LB + SB		LB	SB	ND	PD	LB + SB		
Singletons	670	8	0	8	678	1.2	1059	19	2	21	1078	1.9	0.26
Twins	308	18	8	26	326	8.0	433	16	8	24	449	5.3	0.18
Triplets	17	1	1	2	18	11.1	15	0	0	0	15	—	—
Multiples (total)	325	19	9	28	344	8.1	448	16	8	24	464	5.2	0.11
Total	995	27	9	36	1022	3.5	1507	35	10	45	1542	2.9	0.42

LB, live birth; SB, still birth; ND, neonatal death ≤ 7 days; PD, perinatal death. The perinatal death rate = sum of number of stillborns and neonatal deaths divided by sum of all live births and stillborns.
[a]All data represent number of cases (children), unless stated otherwise.
***P* values for univariate analysis comparing perinatal death rates among PGD and ICSI children.

Table II Birthweight, height and head circumference in PGD and ICSI singletons.

	PGD children	ICSI children	*P**
Birthweight (gr)	3262.8 ± 543.5 (660)	3236.5 ± 583.2 (1048)	0.352
Birthweight (sds)	−0.34 ± 1.27 (655)	−0.42 ± 1.41 (1045)	0.099
Birth height (cm)	49.79 ± 2.70 (626)	49.58 ± 3.13 (1023)	0.162
Birth height (sds)	−0.23 ± 1.29 (626)	−0.33 ± 1.41 (1023)	0.136
Birth head circumference (cm)	34.30 ± 1.64 (557)	34.21 ± 1.90 (910)	0.420
Birth head circumference (sds)	−0.22 ± 1.23 (557)	−0.31 ± 1.43 (910)	0.249

sds, standard deviation score.
Data are mean ± SD, (*n*).
**P* values for univariate analysis (unadjusted) comparing a variable among PGD and ICSI children.

Table III Birthweight, height and head circumference in PGD and ICSI multiples.

	PGD children	ICSI children	*P**
Birthweight (gr)	2299.8 ± 581.1 (307)	2248.1 ± 582.1 (428)	0.235
Birthweight (sds)	−2.74 ± 1.62 (303)	−2.86 ± 1.69 (426)	0.315
Birth height (cm)	45.46 ± 4.07 (281)	45.30 ± 3.87 (397)	0.609
Birth height (sds)	−2.22 ± 1.76 (281)	−2.31 ± 1.68 (397)	0.525
Birth head circumference (cm)	32.35 ± 2.38 (230)	32.13 ± 2.36 (333)	0.277
Birth head circumference (sds)	−1.76 ± 2.17 (230)	−1.93 ± 2.19 (333)	0.366

sds, standard deviation score.
Data are mean ± SD, (*n*).
**P* values for univariate analysis (unadjusted) comparing a variable among PGD and ICSI children.

height and head circumference at birth for neonates born after PGD compared with ICSI (Tables II and III). Mean gestational age at birth for PGD singletons (38.7 ± 1.9 weeks), twins (35.3 ± 1.6 weeks) and triplets (32.2 ± 3.7 weeks), and number of prematurely (<37 weeks) born PGD singletons ($n = 71$) and multiples ($n = 157$) showed no differences compared with their ICSI counterparts (all *P*-values > 0.05). Twenty-one ICSI and 4 PGD singletons versus 67 ICSI and 31 PGD multiples were born very prematurely (<32 weeks) which is not significant ($P = 0.056$ and $P = 0.43$ for singletons and multiples, respectively). Admission after delivery to the neonatal intensive care unit (107 PGD singletons, 186 PGD multiples, 197 ICSI singletons, 273 ICSI multiples) was comparable for PGD and ICSI groups for singletons ($P = 0.23$) as well as multiples ($P = 0.17$).

Maternal age within the PGD group was higher compared with the ICSI group ($P < 0.001$). Parity was higher within the ICSI group ($P < 0.001$). PGD mothers presented on average with a lower pre-pregnancy BMI ($P = 0.002$). More pregnancy complications (placental complications, thyroid pathology, gestational diabetes, pregnancy-induced hypertension, pre-eclampsia and premature contractions) were registered for PGD women ($P = 0.001$) (Table IV). Table IV shows that ICSI mothers smoked cigarettes more frequently during pregnancy ($P = 0.038$) while intake of alcohol was higher for PGD mothers ($P = 0.034$).

Multivariable analyses exploring the impact of PGD and ICSI, simultaneously adjusting for maternal age, pre-pregnancy BMI, parity, nicotine abuse, intake of alcohol and complications during pregnancy, did not alter the findings of the unadjusted analyses listed in Tables II and III.

Twenty-three live born PGD children (2.3%; 95% CI 1.5–3.4) and 40 live born ICSI children (2.7%; 95% CI 2.0–3.6) presented major malformations (OR 0.868; 95% CI 0.492–1.496; $P = 0.69$).

Major genital malformations were recorded for seven PGD neonates (0.7%) and nine ICSI (0.6%) neonates ($P = 0.80$). Major genital malformations within the PGD group included intrauterine torsion of testicles in one child, hypospadias in four children, and testicular atrophy in two children. Within the ICSI group, five children presented cryptorchid testicles and four children had hypospadias (Supplementary data, Table SI). The total (stillborn and live born) major malformation rate was comparable in the PGD group (2.6%) and ICSI group (3%) ($P = 0.63$). More specifically, four stillborns

Table IV Characteristics of the women, and their pregnancies, who underwent PGD or ICSI.

	PGD mothers	ICSI mothers	*P**
Maternal age (years)[a]	33.2 ± 4.6	32.0 ± 4.2	**<0.001**
Parity mother (1/>1)	75/25	66/34	**<0.001**
Pre-pregnancy BMI[a] (kg/m^2)	22.7 ± 3.5 (559)	23.3 ± 4.3 (951)	**0.002**
Pregnancy complications (%)	55	47	**0.001**
Cigarette smoking in pregnancy (%)	5	7	**0.038**
Alcohol intake during pregnancy (%)	10	7	**0.034**
Duration of pregnancy (weeks)[a]			
Total group	38.1 ± 2.6 (700)	38.0 ± 2.8 (1276)	0.761
Singletons	38.7 ± 1.9 (573)	38.7 ± 2.2 (1048)	0.747
Multiples	35.0 ± 3.1 (228)	35.1 ± 3.2 (127)	0.752

The bold values indicate statistical significant values.
[a]Data are Mean ± SD, (*n*).
**P* values for univariate analysis comparing a variable among PGD and ICSI children.

(total number: 27) conceived after PGD and seven stillborns (total number: 35) conceived after ICSI presented major malformations.

Discussion

Since the cleavage-stage biopsy technique is increasingly applied and large controlled clinical perinatal outcome studies are still uncommon, it is important to collect follow-up data on children conceived by this method in order to assess their outcome. The main objective of this study was to determine whether the embryo biopsy procedure for PGD purposes affects health outcome of children at birth. In this study, neonatal data of a large cohort of 995 live born children born after embryo biopsy procedure (PGD) were compared with outcome data of 1507 live born children conceived by the same ART procedure but without blastomere biopsy (ICSI). The main findings of this study are that embryo biopsy neither adds significant risk to the perinatal health of newborn PGD singletons and multiples, nor does it change the risk of major malformations. Under the given case numbers and assuming an alpha level of 0.05, our study achieves 90% power to detect an OR of 2.0. Alternatively, the given case numbers can detect an OR of 1.839, assuming an alpha of 0.05 and a power of 80%. A further strength of our study is that it provides comparative data on consistent perinatal follow-up in the largest PGD children cohort published.

No differences regarding gestational age, incidence of preterm births and weight, length and head circumference data at birth were found between the singletons and multiples who were conceived either in association with cleavage-stage biopsy for PGD or those without biopsy, however ICSI multiples showed a higher incidence of low birthweight (<2500 g). Some evidence indicates that pregnancies following fertility treatment in infertile couples carry an increased incidence of preterm delivery, lower birthweight and other adverse birth outcomes. Infertile women are at higher risk of adverse birth outcomes even if they conceive without treatment i.e. infertility as an independent factor increases the risk of adverse obstetric outcome (Basso and Baird, 2003; Romundstad *et al*., 2008; Wisborg *et al*., 2010). Taking into account that the ratio of infertile couples is higher within the ICSI cohort compared with the PGD group (the potentially more fertile group), this study showed no increased risk of preterm delivery but significantly more ICSI multiples with a birthweight under 2500 g, which might be explained by the fertility status of their parents.

In 2009, we reported no statistically significant differences for the gestational age and the auxological outcome in a small group of 102 neonates born after PGD compared with ICSI (Desmyttere *et al*., 2009), however, this study had a small sample size and low power. Tur-Kaspa *et al*. (2005) also mentioned comparable term and birthweight data to IVF/ICSI pregnancies in an abstract reporting on the outcome of 480 PGD children. Banerjee *et al*. (2008) on the other hand, described a lower gestational age, more preterm births and lower birthweight in 49 PGD children compared with neonates born after natural conception. Liebaers *et al*. (2010) analysed the mean gestation length and birthweight parameters of 581 children born after PGD included in the present study but compared this to historical data on ICSI children. More PGD singletons and multiples were born prematurely and significantly more PGD multiples presented low birthweight (Liebaers *et al*. 2010). In the present study, more ICSI multiples presented low birthweight compared with their PGD counterparts. Perinatal death rates, as previously reported by Liebaers *et al*. (2010) were much higher in post-PGD multiple pregnancies (11.7%) compared with ICSI multiple pregnancies (2.5%) but were similar for PGD (1.03%) versus ICSI (1.3%) singletons. In this significantly larger analysis, however, including the series reported earlier by Liebaers *et al*. (2010), we found, reassuringly so, no difference in perinatal death rates between PGD singletons (1.2%) and PGD multiples (8.1%) in comparison with ICSI singletons (1.9%) and ICSI multiples (5.2%), respectively. The absolute perinatal death rate in multiples remains, however, unreassuringly high, justifying the restriction of numbers of embryos for transfer, even in PGD treatment. A previous study showed no difference in pregnancy rates between single and higher order embryo transfer in PGD (Donoso *et al*., 2007).

The major malformation rate is comparable in PGD and ICSI live borns, which is in accordance with the limited data in the literature although it is difficult to compare malformation rate because different definitions and examination methods are used. Strom *et al*. (2000) reported major malformations in 2 of 109 children (1.8%) born after PGD by polar body removal. A major malformation rate of between 1.7 and 1.9% was calculated from data in two abstracts (Horwitz *et al*., 2005; Tur-Kaspa *et al*., 2005) which reported on an overlapping group of 480 and 576 PGD children, respectively. Liebaers *et al*. (2010) reported total rates of major malformations of 2.13% for PGD children and 3.38% for ICSI children. Banerjee *et al*. (2008) reported two children with major malformations from 49 children (4.1%).

The present study does not indicate that the cleavage-stage biopsy procedure adds significant risks of major birth defects compared with the ICSI procedure. Several studies in the literature suggest that children born after ART are at increased risk of birth defects compared

with natural conceptions (Rimm et al., 2004; Hansen et al., 2005; Lie et al., 2005). Therefore the risk of major malformations in PGD children is presumably higher compared with the risk for children born after a natural conception, however this cannot be confirmed in this particular study as we made no comparison with children born after natural conception. To date, no studies have compared the outcome of major malformations in PGD children with children conceived naturally.

In conclusion, neonatal outcome parameters (auxological data, gestational age, neonatal admission and major malformations) were similar in singletons and multiples born after embryo biopsy for PGD compared with a control cohort of singletons and multiples without embryo biopsy. However, multiples born following PGD had a reduced risk of low birthweight than multiples born following ICSI. Major malformation and perinatal death rates revealed reassuring findings.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors' roles

F.D.S., I.L., M.B., P.H., S.D. and W.P. were involved in study design, acquisition of data, drafting and critical discussion. P.H. and S.D. analysed the data. C.S. and M.D.R. performed the chromosome and DNA analysis and gave their critical advice.

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