Two-year auxological and medical outcome of singletons born after embryo biopsy applied in preimplantation genetic diagnosis or preimplantation genetic screening

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BACKGROUND: Embryo biopsy is an essential but invasive procedure to perform preimplantation genetic diagnosis (PGD) or preimplantation genetic screening (PGS). The major objective of this study was to determine whether embryo biopsy might cause post-natal growth restriction.

METHODS: We compared growth data and physical findings at birth and 2 years for singletons born either after PGD/PGS (n = 70), ICSI (n = 70) or natural conception (NC) (n = 70). Children were matched for gender, maternal educational level, mother tongue and birth order.

RESULTS: No significant differences were found between the three groups regarding weight, height and head circumference standard deviation scores (SDS) at birth and at age 2 years, although the PGD/PGS children tended to have a lower birthweight compared with the NC children. At 2 years, the mean BMI SDS in PGD/PGS children was significantly lower compared with NC children (P = 0.005). PGD/PGS children were more frequently born after Caesarian section than ICSI children, but had no more congenital malformations, hospital admissions and surgical interventions compared with ICSI and NC children.

CONCLUSIONS: Singleton children at age 2 years born after embryo biopsy applied in PGD/PGS present a similar post-natal linear growth compared with ICSI and NC children. PGD/PGS singletons appear not to be at higher risk for congenital malformations and surgical interventions during the first 2 years of life. To date, there have been no observable detrimental effects of the PGD/PGS procedure on children.

Key words: embryo biopsy / PGD / preimplantation genetic screening / post-natal growth / children
Introduction

Preimplantation genetic diagnosis (PGD) allows us to determine specific monogenic defects, chromosomal imbalances and gender of the embryo obtained through IVF (Lissens et al., 1996; Sermon et al., 2004). In the recruitment period of this study, preimplantation genetic screening (PGS) with enumeration of chromosomes or aneuploidy screening was applied to improve the effectiveness of assisted reproduction treatment (ART) in patients at low risk of transmitting a genetic disease, but with an increased incidence of embryonic numerical chromosome abnormalities, repeated abortions, multiple failed IVF cycles or advanced maternal age (Shenfield et al., 2003; Sermon, 2006). Today, however, it is a matter of controversy whether PGS improves the outcome of ART (Mastenbroek et al., 2007). ICSI is used in ART to reduce the risk of fertilization failure in comparison to IVF and to minimize the risk of contamination by DNA in PCRs. Embryo biopsy, with the removal of one or two (exceptionally more) blastomeres, is performed from the 6- to the 8-cell cleavage stage onward (Sermon, 2006). PGD/PGS is a more invasive technique compared with ICSI and regular IVF. To date, studies investigating neonatal outcome after PGD/PGS have reported reassuring findings. Strom et al. (2000) described the neonatal outcome in 109 PGD children and found no higher rate of congenital defects. Sermon et al. mentioned in the European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium report that characteristics at birth were comparable to those of ICSI babies (Bondeulle et al., 2002; ESHRE, 2007). Keymolen et al. (2007) observed no major malformations in 25 live born children after PGD for cystic fibrosis. Banerjee et al. assessed 39 PGS and 10 PGD children and found their birthweight was significantly lower in comparison to natural conception (NC) controls and that growth parameters assessed at the mean age of 18 months were within the normal range (Banerjee et al., 2008).

Since subtle somatic, neurological and psychological abnormalities may only become apparent as the children mature, a case–control study was set up at our centre focusing on a cohort of 2-year-old children born after PGD/PGS. These children had assessments at ages 2 months and 1 year. In this study, a third assessment was performed within a prospective research. Detailed socio-demographic data were obtained, since these variables can influence growth (Cogswell and Yip, 1995; Kramer et al., 2002). A control group of NC children was used and, in addition, an ICSI control group was included to determine whether potential differences in children’s outcome were exclusively related to the embryo biopsy.

We report here the auxological and clinical outcome data of this cohort study at 2 years. We additionally investigated whether the body size of children born after biopsy of one blastomere was different from that of children born after biopsy of two blastomeres.

Motor and mental development characteristics of these children were assessed as well and are reported elsewhere (Nekkebroeck et al., 2008).

Materials and Methods

Study participants

Our study population consists of 70 PGD/PGS, 70 ICSI and 70 NC children. The children born after PGD/PGS and ICSI are part of a cohort followed since birth (Bondeulle et al., 2002). PGD/PGS children were eligible if they were singleton, living in Belgium, Caucasian and if their mother tongue was Dutch, French or English. Twins were excluded because of possible confounders (e.g. prematurity, low birthweight) that may interfere with development and clinical outcome (Miceli et al., 2000). The applied biopsy technique through aspiration of blastomeres was the same in all PGD/PGS conceptions (Goossens et al., 2007). Eight PGD/PGS children were born after biopsy of one blastomere, whereas 56 PGD/PGS children underwent a two-blastomere biopsy. Data are omitted for six singletons born after the transfer of two embryos (with a 1-cell biopsy for one embryo and a 2-cell biopsy for the other), as we do not know which embryo developed completely.

PGD/PGS children were enlisted so that at assessment between April 2005 and April 2007 their age would range between 21 and 33 months. Twenty-eight children within the PGD/PGS group were born after PGD and 42 children after PGS. ICSI and NC controls were selected to match PGD/PGS children as closely as possible according to gender, maternal educational level, mother tongue and birth order (having an older sibling or not) (Table 1). NC control children were recruited from the university hospital day care centres (n = 38) and a pediatrician’s practice (n = 32). All selected children were invited and examined at a time when they were not ill.

Of the eligible PGD/PGS children (n = 77), 70% or 91% of the children participated in the study. In order to include 70 ICSI children and 70 NC controls, 74 ICSI and 79 NC families were contacted, resulting in a participation rate of 94.6% in the ICSI group and 88.6% in the NC group.

Recruitment

Before starting PGD/PGS or ICSI, couples were asked to participate in a prospective clinical follow-up study and a written informed consent was obtained. Since data on pregnancy and birth were obtained through written questionnaires that were sent to the couples during the 6th month of pregnancy, the research nurse contacted the PGD/PGS and ICSI parents as soon as possible when the questionnaires, returned after the birth of their child, reached us. The parents were invited for a clinical examination of their child at the age of 2 months. If an appointment could not be made, the parents were motivated to continue the follow-up study at the age of 1 and 2 years.

Parents of NC children were invited by letter containing detailed information of the study and asking for consent to participate. The coordinating nurse from the day care centres and the pediatrician planned the appointments for the NC children.

Study procedure

All questionnaires were filled out by the parents of the PGD/PGS, ICSI and NC children on the examination day of their child at age 2 years. Systematically, all pregnancy and neonatal records were double checked with the medical files from hospitals and free healthcare preventive organizations ('Kind en Gezin' and 'Oeuvre Nationale des Enfants' who register birth data—e.g. auxological parameters, Apgar score, neonatal admissions (‘Kind en Gezin’ and 'Oeuvre Nationale des Enfants’ who register birth data—e.g. auxological parameters, Apgar score, neonatal admission—from all children) with the consent of the parents. NC controls were examined at their day care centre or at the pediatrician’s practice and in the presence of at least one of the parents. PGD/PGS and ICSI children were examined at the Centre for Medical Genetics. The examination consisted first of an assessment of the mental and motor development by a psychologist and afterwards a general physical examination was performed by the pediatrician.

Socio-demographic parameters of the family

Information about the residence of the child as well as the marital status, ethnic origins, education level of the parents and the percentage of their time spent at work were obtained.
Medical history of the father, mother and pregnancy

The height and weight of both biological parents were recorded as reported by both parents. Information about the mother included her age at birth of the child, menarche, parity, health during pregnancy, her use of medication, alcohol and cigarettes during pregnancy and weight gain during pregnancy.

Medical history of the child

Perinatal data, i.e. gestational age, birthweight, birth height, head circumference at birth, Apgar score, breastfeeding and reason and duration of admission to a neonatal care unit, were collected. Childhood medical history consisted of hospital admissions, surgical interventions and chronic illnesses. Chronic illness was defined as a disorder of at least 3 months in duration in any 1 year, which required treatment or interfered with daily functioning. Reason, frequency and duration of hospital admissions were noted. Intake of medication for more than 3 weeks, remedial treatment and complementary examinations were noted.

Biometric data and physical examination of the child

All children had a thorough physical examination, performed by a pediatrician who was blinded to the type of ART. Blinding towards conception mode was not possible for NC children, who were examined at the day care centre or at a pediatrician’s practice. Biometric data, such as weight, height, head circumference, left mid-upper arm circumference and waist circumference, were collected with standard equipment and according to a standardized procedure (www.vub.ac.be/groeicurven). Weight, height, head and arm circumference standard deviation scores (SDS) were calculated from the recent Flemish growth survey in 2004 (www.vub.ac.be/groeicurven). Physical examination included checking for possible major and minor anomalies. Skin defects including café-au-lait spots, haemangioma, congenital naevi and eye movement disorders (covertest) were also noted. Malformations were classified according to criteria previously defined at our centre. A major malformation causes functional impairment and/or requires surgical correction (Bonduelle et al., 1995, 1999, 2002). The remaining malformations were classified as minor anomalies according to a checklist for minor congenital defects (Aase, 1990). Internal standardized guidelines for major and minor defects were also used (Bonduelle et al., 2002).

Sample size calculation and feasibility of the study

A power analysis showed that it is possible with 70 children in each group to detect a difference in height of 0.94 SDS, or 3.4 cm, between the groups at the age of 2 years for an α level of 0.05 and 90% power. With 70 children in each group, and assuming a 2.6% malformation rate in the control group, it is possible to detect only a 1.34 times higher malformation rate in the PGD/PGS group for an α level of 0.05 and 80% power. A sample size of 670 children in each group is required to detect a doubling in major malformations for an α level of 0.05 and 80% power.

Statistical analysis

The study power analysis and sample size calculations were validated with nQuery Advisor version 5.0 (Statistical Solutions Ltd, Ireland). Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) 15.0 for Windows. Results are expressed as mean ± SD. Univariate analyses of variance were performed to determine differences in mean scores of continuous variables between children conceived after PGD/PGS, ICSI and NC controls. If a group effect was identified, post hoc, least significant difference (LSD) or Tukey’s test was conducted to determine which group differed significantly from each other. Categorical variables were analysed using χ². The between-group differences for anthropometric outcomes at birth were analysed using the analysis of variance (ANOVA) model incorporating the mode of conception group, gender, parity, pregnancy complications, maternal intake of alcohol and tobacco as fixed factor effect and maternal pregnancy weight gain, maternal age, gestational age, mode of delivery, the BMI and height of both the parents as covariates. The same model incorporating the mode of conception and gender as fixed factor effect, and the gestational age, birth height and birthweight SDS, age of the child at examination, duration of breastfeeding, number of episodes of chronic illness, the BMI and height of both the parents as covariates, was used to analyse the 2-year anthropometric outcomes.

Table I Socio-demographic characteristics of the families of singleton children born after PGD/PGS, ICSI or NC

<table>
<thead>
<tr>
<th></th>
<th>PGD/PGS, n = 70</th>
<th>ICSI, n = 70</th>
<th>NC, n = 70</th>
<th>Test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at child birth* (years)</td>
<td>34.2 ± 4.6</td>
<td>33.6 ± 4.0</td>
<td>29.9 ± 4.3</td>
<td>F = 20.76</td>
<td>0.000</td>
</tr>
<tr>
<td>Paternal age at child assessment* (years)</td>
<td>38.0 ± 5.1</td>
<td>39.1 ± 6.0</td>
<td>34.1 ± 5.6</td>
<td>F = 15.75</td>
<td>0.000</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mothers: high/medium/low</td>
<td>50/17/3</td>
<td>49/20/1</td>
<td></td>
<td>χ²(4) = 1.26</td>
<td>0.868</td>
</tr>
<tr>
<td>Fathers: high/medium/low</td>
<td>42/24/2</td>
<td>40/21/8</td>
<td>46/20/4</td>
<td>χ²(4) = 4.83</td>
<td>0.305</td>
</tr>
<tr>
<td>Type of childcare</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At home with one parent</td>
<td>22</td>
<td>10</td>
<td>8</td>
<td>χ²(8) = 16.66</td>
<td>0.034</td>
</tr>
<tr>
<td>With family</td>
<td>9</td>
<td>14</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day care centre</td>
<td>26</td>
<td>35</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child Minder</td>
<td>12</td>
<td>10</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>School</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth order</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eldest or only child/youngest or middle child</td>
<td>49/21</td>
<td>49/21</td>
<td>46/24</td>
<td>χ²(2) = 0.40</td>
<td>0.920</td>
</tr>
</tbody>
</table>

Values are n unless otherwise stated.
*Mean ± SD.
The mean group pairwise comparisons of anthropometric outcomes were calculated based on estimated marginal means from the ANCOVA model and were statistically not significant. A significance level of 0.05 was accepted throughout.

**Ethics**

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

**Results**

The results for socio-demographic data, neonatal and 2-year growth parameters, and determinants of the children’s growth are presented in Tables I–IV, respectively.

**Socio-demographic data**

Seventy children were assessed in each conception group. As the ICSI and NC controls were selected to match the PGD/PGS cases as closely as possible for gender, maternal educational level, birth order and mother tongue, no differences were found between the three conception groups for these characteristics. Gestational age and the number of children born after less than 37 weeks of gestation were the same in the three conception groups. Although probably not clinically relevant, there was a small difference in the average age of the children at the time of assessment between the conception groups, with PGD/PGS children being slightly but significantly younger than the ICSI and NC children (post hoc LSD: PGD/PGS < ICSI \( P = 0.002 \) and PGD/PGS < NC \( P = 0.012 \) (Table III). NC mothers were significantly younger at the time of delivery than the mothers in the PGD/PGS and ICSI groups (post hoc LSD: NC < PGD/PGS \( P = 0.000 \) and NC < ICSI \( P = 0.000 \)). NC fathers were also significantly younger at the time of the child assessment compared with the two other conception groups (post hoc Tukey: PGD/PGS < ICSI \( P = 0.059 \) and PGD/PGS < NC \( P = 0.000 \)).

### Table II Neonatal growth parameters in PGD/PGS, ICSI and NC children

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PGD/PGS</th>
<th>ICSI</th>
<th>NC</th>
<th>Test</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight (g)</td>
<td>3168.4 ± 532.2 (70)</td>
<td>3164.6 ± 626.5 (70)</td>
<td>3336.2 ± 502.2 (70)</td>
<td>( F = 2.173 )</td>
<td>0.116</td>
</tr>
<tr>
<td>Birth height (cm)</td>
<td>49.2 ± 2.5 (68)</td>
<td>49.2 ± 3.0 (69)</td>
<td>50.1 ± 2.7 (70)</td>
<td>( F = 2.252 )</td>
<td>0.108</td>
</tr>
<tr>
<td>Birth head circumference (cm)</td>
<td>34.3 ± 1.4 (63)</td>
<td>34.0 ± 1.9 (59)</td>
<td>34.1 ± 1.6 (42)</td>
<td>( F = 0.473 )</td>
<td>0.624</td>
</tr>
<tr>
<td>Birthweight SDS</td>
<td>-0.33 ± 1.00 (70)</td>
<td>-0.33 ± 1.1 (70)</td>
<td>-0.19 ± 0.98 (70)</td>
<td>( F = 0.471 )</td>
<td>0.625</td>
</tr>
<tr>
<td>Birth height SDS</td>
<td>-0.17 ± 1.00 (68)</td>
<td>-0.76 ± 1.6 (69)</td>
<td>0.08 ± 1.27 (70)</td>
<td>( F = 0.834 )</td>
<td>0.436</td>
</tr>
<tr>
<td>Birth head circumference SDS</td>
<td>0.01 ± 0.90 (63)</td>
<td>-0.1 ± 0.94 (59)</td>
<td>-0.08 ± 1.03 (42)</td>
<td>( F = 1.333 )</td>
<td>0.266</td>
</tr>
</tbody>
</table>

Data are mean ± SD (n).

SDS, standard deviation scores.

### Table III Growth parameters at age 2 years in PGD/PGS, ICSI and NC children

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PGD/PGS</th>
<th>ICSI</th>
<th>NC</th>
<th>Test</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age child (years)</td>
<td>2.2 ± 0.2 (70)</td>
<td>2.3 ± 0.0 (70)</td>
<td>2.3 ± 0.2 (70)</td>
<td>( F = 5.714 )</td>
<td>0.004</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>13.0 ± 1.3 (70)</td>
<td>13.4 ± 1.7 (70)</td>
<td>13.5 ± 1.5 (70)</td>
<td>( F = 2.432 )</td>
<td>0.090</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>90.6 ± 5.2 (70)</td>
<td>91.9 ± 3.9 (70)</td>
<td>91.4 ± 3.4 (70)</td>
<td>( F = 1.505 )</td>
<td>0.224</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>48.8 ± 1.4 (69)</td>
<td>49.0 ± 1.4 (70)</td>
<td>48.8 ± 1.6 (68)</td>
<td>( F = 0.559 )</td>
<td>0.573</td>
</tr>
<tr>
<td>Left arm circumference (cm)</td>
<td>15.7 ± 1.2 (67)</td>
<td>15.9 ± 1.3 (66)</td>
<td>16.0 ± 1.4 (67)</td>
<td>( F = 0.834 )</td>
<td>0.436</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>48.7 ± 3.5 (66)</td>
<td>48.0 ± 3.1 (67)</td>
<td>49.0 ± 3.7 (66)</td>
<td>( F = 1.365 )</td>
<td>0.258</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>0.08 ± 0.84 (70)</td>
<td>0.18 ± 1.03 (70)</td>
<td>0.30 ± 1.00 (70)</td>
<td>( F = 0.974 )</td>
<td>0.379</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.71 ± 0.94 (70)</td>
<td>0.63 ± 1.05 (70)</td>
<td>0.56 ± 1.00 (69)</td>
<td>( F = 0.395 )</td>
<td>0.674</td>
</tr>
<tr>
<td>Head circumference SDS</td>
<td>0.032 ± 0.92 (69)</td>
<td>0.08 ± 0.88 (70)</td>
<td>-0.08 ± 1.03 (68)</td>
<td>( F = 0.496 )</td>
<td>0.609</td>
</tr>
<tr>
<td>Arm circumference SDS</td>
<td>-0.27 ± 0.91 (67)</td>
<td>-0.13 ± 0.92 (66)</td>
<td>-0.09 ± 1.00 (67)</td>
<td>( F = 0.663 )</td>
<td>0.516</td>
</tr>
<tr>
<td>Waist circumference SDS</td>
<td>0.48 ± 1.02 (66)</td>
<td>0.24 ± 0.94 (67)</td>
<td>0.50 ± 1.12 (66)</td>
<td>( F = 1.324 )</td>
<td>0.268</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>-0.57 ± 1.11 (67)</td>
<td>-0.31 ± 1.02 (67)</td>
<td>-0.06 ± 1.04 (69)</td>
<td>( F = 4.049 )</td>
<td>0.019</td>
</tr>
<tr>
<td>Delta weight SDS</td>
<td>0.41 ± 1.15 (70)</td>
<td>0.52 ± 0.98 (70)</td>
<td>0.49 ± 1.08 (70)</td>
<td>( F = 0.191 )</td>
<td>0.826</td>
</tr>
<tr>
<td>Delta height SDS</td>
<td>0.88 ± 1.09 (68)</td>
<td>0.71 ± 1.13 (69)</td>
<td>0.48 ± 1.16 (69)</td>
<td>( F = 2.182 )</td>
<td>0.115</td>
</tr>
<tr>
<td>Delta head circumference SDS</td>
<td>-0.01 ± 0.95 (62)</td>
<td>0.15 ± 0.94 (59)</td>
<td>0.11 ± 1.14 (40)</td>
<td>( F = 0.416 )</td>
<td>0.660</td>
</tr>
</tbody>
</table>

Data are mean ± SD (n).

*Delta weight, height, head circumference is the difference in weight, height, head circumference SDS between age 2 years and at birth.*
Most of the children in all three conception groups lived in two-parent families consisting of both biological parents, although more NC children had parents who were divorced in comparison to the PGD/PGS group (i.e. 37/33 vs. 24/24). Maternal pregnancy parameters influencing prenatal growth such as lifestyle, pregnancy complications, parity, weight gain during pregnancy and maternal anthropometrical determinants such as pre-pregnancy BMI and stature are shown in Table IV. PGD/PGS offspring were more often born after Caesarian section compared with ICSI babies (i.e. 21/24 vs. 11/11). Eight PGD/PGS children were born after biopsy of one blastomere, whereas 56 PGD/PGS children underwent a two-blastomere biopsy. Anthropometrical data at birth were comparable for these groups (data not shown).

Prenatal and birth data

Anthropometrical data at birth, i.e. weight, height and head circumference data and SDS, were comparable for the three conception groups (Table II). Maternal pregnancy parameters influencing prenatal growth such as lifestyle, pregnancy complications, parity, weight gain during pregnancy and maternal anthropometrical determinants such as pre-pregnancy BMI and stature are shown in Table IV. PGD/PGS offspring were more often born after Caesarian section compared with ICSI babies (i.e. 21/24 vs. 11/11). Eight PGD/PGS children were born after biopsy of one blastomere, whereas 56 PGD/PGS children underwent a two-blastomere biopsy. Anthropometrical data at birth were comparable for these groups (data not shown).

Data at 2 years

Post-natal anthropometrical data, i.e. weight, height, head circumference, waist and arm circumference data and SDS recorded at the moment of the study, were comparable for the three conception groups (Table III). Relevant paternal, maternal and medical history characteristics are shown in Table IV.
In PGD/PGS children, the mean BMI SDS was statistically significantly lower compared with NC children (post hoc LSD PGD/PGS < NC P = 0.005).

Admission to a neonatal ward was comparable in the three conception groups. PGD/PGS children did not experience more hospital stays for medical reasons than the ICSI and NC groups. A surgical intervention was performed in 5/70 PGD/PGS children compared with 12/70 ICSI and 4/70 NC children [x²(2) = 6.03, P = 0.049]. Regarding child hospital admission for surgical reasons, no significant difference was noted when comparing PGD/PGS with ICSI [x²(1) = 3.281, P = 0.070] and PGD/PGS with NC [x²(1) = 0.119, P = 0.730]. Two NC children required genito-urinary surgery, one child underwent a bilateral orchidopexy and another child had a circumcision, compared with no ICSI children and one PGD/PGS child (circumcision).

Comparable rates of chronic diseases and chronic use of medication were reported (Table IV).

PGD/PGS children underwent significantly more complementary investigations (e.g., radiological examinations, pH-metry, isotopic investigations) compared with ICSI children [x²(1) = 4.24, P = 0.039] and NC children [x²(1) = 7.75, P = 0.005]. The number of complementary investigations with an abnormal result was not significantly different between the groups. Growth parameters of the PGD/PGS children born after biopsy of one blastomere were comparable to those after biopsy of two blastomeres (data not shown).

### Major and minor malformations

Two PGD/PGS children, one ICSI child and two NC children had major malformations. Within the PGD/PGS group, one child suffered from a ventricular septum defect and another child had hemivertebrae with gibbus. One child from the ICSI group born at 39 weeks underwent surgery for an inguinal hernia. Within the NC group, a surgical intervention was performed in one child with a transposition of the great arteries and in another child with bilateral cryptorchidism.

We recorded 24 PGD/PGS children, 23 ICSI children and 13 NC children with one or more minor malformations [x²(2) = 5.18 P = 0.075]. The overall incidence of minor birth defects was not significantly different between the groups. Skin anomalies (naevi, hemangioma, café au lait spot) were the most frequently observed minor malformations in the three groups: 18 ICSI children (25.7%), 14 PGD/PGS children (20%) and 10 NC children (14.3%). Other minor anomalies such as overriding toes, heart murmur or prominent ears were rare.

### Discussion

The aim of this study was to compare growth data for 2-year-old singletons born after PGD/PGS, ICSI and NC. In addition, within the PGD/PGS group, growth parameters were compared for children conceived after biopsy of one or two blastomeres.

Until now, it is mainly the neonatal growth data of children born after PGD/PGS which have been reported, and the potential effect of the number of blastomeres biopsied has not been evaluated. We reported that growth characteristics within the PGD/PGS group after biopsy of one or two blastomeres were comparable. Given the small number of children studied after one-blastomere biopsy (8) and two-blastomere biopsy (56), analyses of larger samples of children are mandatory to confirm this finding.

The PGD/PGS singletons in our study tended to have a lower birthweight and gestational age compared with the NC children, although this difference did not reach statistical significance. Meta-analyses have clearly shown that singletons born after ART are at risk of lower birthweight (Jackson et al., 2004). Sermon et al. reported that biometric parameters at birth for PGD/PGS singletons were comparable to those of ICSI babies (ESHRE, 2007). Banerjee et al. (2008) described a lower gestational age, more preterm births and lower birthweight in 49 PGD/PGS children compared with NC children.

We documented that the anthropometrical data, i.e., height and weight of 2-year-old PGD/PGS children, were comparable to those of the ICSI and NC groups. The only available data on older children born after PGD/PGS compared with NC children are published by Banerjee et al. (2008) and show that growth parameters assessed at a mean age of 18 months were within a normal range. Singletons as well as twins born mainly after PGS (80%) were included in the small study group reported by Banerjee et al. (2008).

An unexpected finding of our study was that the BMI and arm circumference SDS, although within normal limits, were lower in PGD/PGS children compared with NC children, although not significantly so for the arm circumference. These parameters had never previously been analysed in PGD/PGS children. Several predictors of birthweight such as parity, gestational age, maternal pre-pregnancy BMI and weight gain during pregnancy were comparable for the studied groups. Premature contractions and older maternal age were more common in the PGD/PGS group, both known to be more frequent in mothers undergoing ART, and possibly related to lower birthweight. It is not unlikely that higher maternal age in the PGD/PGS group may be associated with higher income, although we matched for maternal educational level. If income is associated with nutrition, this may explain differences in body composition. PGD/PGS procedures are not only available to wealthier couples. Patients of all socio-economic backgrounds have access to ART in Belgium as a result of changes in reimbursement since 2003. We speculate that the awareness of ‘healthy food consumption’ might be higher among PGD/PGS parents, which may influence auxological parameters during childhood.

We found, at birth and 2 years of age, no higher prevalence of congenital malformations and post-natal diseases or surgical interventions in PGD/PGS children compared with ICSI and NC children. Since there is a lack of power to detect major malformations, it is too early to draw final conclusions.

PGD/PGS children were more often born after Caesarian section compared with ICSI babies, and they underwent more complementary examinations (with normal results) compared with NC and ICSI babies; however, this was not associated with more neonatal problems. Precautionary measures for ‘specially conceived’ children can probably explain these findings. This study did not find an increased rate of medical interventions, as measured by hospitalizations and operations, in PGD/PGS children compared with ICSI and NC children.

The set-up of this study made it possible to avoid several potential biases, such as participation bias, which are frequently encountered in studies evaluating growth and medical outcome of children conceived after different interventions. Participation rates were high in all three conception groups so that participation bias was reduced to a minimum. Socio-demographic confounders were eliminated as much as possible by case control matching for maternal educational level, birth rank, gender and mother tongue. The strength of this study is that all children...
were examined by the same pediatrician in a standardized way. However, bias related to different ways of recruitment has not been completely excluded. Although all ART children at our centre are part of a longitudinal follow-up cohort and therefore receive questionnaires at several time periods, only information gathered at the moment of examination at age 2 years is used for comparison. Nevertheless, a recall bias cannot be excluded since ART parents are more familiar with questionnaires than those in the NC group. The recruitment of children in a day care setting may indicate that they are ‘healthy’ and less likely to suffer from severe pathology/malformations. This positive selection could lead to an underestimation of the outcome in the PGD/PGS and ICSI group since the NC children would appear ‘more healthy’.

Furthermore, the ability to detect major malformations with our sample size is very limited. An adequate number of children should be accumulated to provide enough statistical power to assess the long-term effects of embryo biopsy. Twins were excluded from this study because of possible interference of prematurity and low birthweight with anthropometrical and malformations outcome. Further research on the outcome of PGD/PGS twins compared with ICSI and NC twins is mandatory.

In conclusion, growth and medical outcome in 2-year-old singleton children born after PGD/PGS when compared with ICSI and NC children revealed reassuring findings. To date, we have found no difference in major malformation rate in a very limited number of children. PGD/PGS singletons appear not to be at higher risk of growth retardation compared with ICSI and NC singletons. Children born after PGD/PGS present a similar early linear post-natal growth compared with their ICSI and NC counterparts. BMI SDS was lower in PGD/PGS children compared with NC children, at least during the first 2 years of life.

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