Human Reproduction Open, pp. 1–7, 2018
doi:10.1093/hropen/hoy013

human reproduction open

ORIGINAL ARTICLE

Body composition and blood pressure in 6-year-old singletons born after pre-implantation genetic testing for monogenic and structural chromosomal aberrations: a matched cohort study

F. Belva [1,*], M. Roelants[2], S. Kluijfhout[1], C. Winter[1], F. De Schrijver[1], S. Desmyttere[1], M. De Rycke[1], H. Tournaye[3], I. Liebaers[1], and M. Bonduelle[1]

[1]Centre for Medical Genetics, Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium [2]Department of Public Health and Primary Care, Environment and Health/Youth Health Care, KU Leuven, Kapucijnenvoer 35, 3000 Leuven, Belgium [3]Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium

*Correspondence address. Centre for Medical Genetics, Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium. Tel: +32 477 6071; E-mail: Florence.belva@uzbrussel.be orcid.org/0000-0003-2358-8014

Submitted on May 7, 2018; resubmitted on July 19, 2018; editorial decision on August 10, 2018; accepted on August 22, 2018

STUDY QUESTION: Does Day 3 embryo biopsy for pre-implantation genetic testing for monogenic (PGT-M) and structural chromosomal aberrations (PGT-SR) affect body composition and blood pressure readings of 6-year-old singletons?

SUMMARY ANSWER: This study of 87 PGT-M and PGT-SR conceived singletons showed no differences in anthropometric measurements and blood pressure readings in comparison with a matched cohort of peers born after ICSI without embryo biopsy.

WHAT IS KNOWN ALREADY: While neonatal outcomes after PGT conception have been found comparable to those after ICSI without embryo biopsy, only a few studies have reported outcomes after PGT at older ages. Moreover, embryo biopsy is also applied in couples who opt for PGT-M and PGT-SR and hence are not necessarily infertile. Health parameters and in particular body composition data in this group of children are lacking.

STUDY DESIGN, SIZE, DURATION: This single-centre matched-pair cohort study evaluated body composition of 6-year-old children born after fresh blastocyst embryo transfer with or without embryo biopsy performed at Day 3 for the purpose of PGT-M and PGT-SR. For each child born after embryo biopsy, a singleton born after transfer of a fresh ICSI embryo at the blastocyst stage and reaching the age of 6 years between May 2011 and June 2017 was matched as closely as possible for gender, age, maternal educational level and birth order.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Anthropometry (weight, height, BMI, skinfold thickness, waist and mid-upper arm circumference) and blood pressure readings in a longitudinally followed cohort of 87 singletons conceived by PGT-M and PGT-SR and a pairwise matched sample of 87 children conceived by ICSI are described. Results are adjusted for current, neonatal and parental characteristics.

MAIN RESULTS AND THE ROLE OF CHANCE: From the 124 eligible PGT-M and PGT-SR families, 110 could be reached of whom 23 refused and 87 (87/110 = 79%) participated. All anthropometric measurements, including *z*-scores of BMI, waist and mid-upper arm circumference, were comparable between the PGT-M and PGT-SR (−0.23; 0.27; 0.17, respectively) and ICSI (−0.29; 0.11; 0.11, respectively) groups (all $P > 0.05$). Furthermore, indices of peripheral (triceps) and central (subscapular) adiposity derived from skinfold thickness were comparable (PGT-M and PGT-SR: 14.7 mm; 11.6 mm and ICSI: 15.5 mm; 11.5 mm) as well as the percentage total body fat mass derived

from these (PGT-M and PGT-SR: 13.7% and ICSI: 13.9%) (all $P > 0.05$). *Z*-scores for blood pressure were also comparable between the PGT and ICSI groups (all $P > 0.05$). Results did not change when adjusted for neonatal (birthweight, birth order), current (age) and parental (smoking during pregnancy, parental BMI) characteristics. Hospitalization rate and surgical intervention rate were not different for PGT-M and PGT-SR children compared to matched peers born after ICSI.

LIMITATIONS, REASONS FOR CAUTION: Although our study describes the largest cohort of singletons born after embryo biopsy worldwide, we were only able to detect moderate differences in anthropometrics and blood pressure with our sample size.

WIDER IMPLICATIONS OF THE FINDINGS: Although Day 3 embryo biopsy followed by blastocyst transfer is not associated with adverse outcomes regarding anthropometry and blood pressure, future studies should focus on outcomes in children born after trophectoderm biopsy and/or transfer of warmed embryos after vitrification.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported by Methusalem grants and by grants from Wetenschappelijk Fonds Willy Gepts; all issued by the Vrije Universiteit Brussel (VUB). All co-authors, except M.B. declared no conflict of interest. M.B. has received consultancy fees from MSD, Serono Symposia and Merck. The Universitair Ziekenhuis Brussel (UZ Brussel) and the Centre for Medical Genetics have received several educational grants from IBSA, Ferring, Organon, Shering-Plough, Merck for establishing the database for follow-up research and organizing the data collection.

Key words: PGD / body mass / child follow-up / IVF/ICSI outcome / embryo biopsy

WHAT DOES THIS MEAN FOR PATIENTS?

Pre-implantation genetic testing for monogenic disorders (PGT-M) and for structural chromosomal aberrations (PGT-SR) involves genetic testing of cells biopsied from *in vitro*-fertilized embryos and the selective transfer of embryos unaffected for the condition under study. It is an option for couples to prevent the transmission of genetic abnormalities to their children. ICSI is applied as the *in vitro* fertilization method, followed by embryo biopsy at Day 3 or Day 5/6; both procedures are invasive for the embryo.

The safety of the ICSI procedure has been assessed in many follow-up studies, but the follow-up after PGT conception with ICSI and embryo biopsy procedures remains limited so far. Previous studies on neonatal outcomes after PGT have found comparable findings to those after ICSI without embryo biopsy. However, few studies have reported outcomes after PGT at older ages. This study looks at whether adding embryo biopsy to the ICSI procedure increases the risk of altered body fat composition or high blood pressure in childhood.

This study suggests that 6-year-old children born after PGT-M/SR with ICSI and embryo biopsy at day three do not differ in terms of BMI, body circumferences, skinfold thicknesses and blood pressure readings from children born after the same treatment protocol but without embryo biopsy. This comparison group, of children born after ICSI without embryo biopsy, resembled the PGT-M/SR group in terms of gender, age, maternal educational level and birth order.

Future studies should focus on outcomes in children born after new treatment protocols, including embryo biopsy performed at Day 5/6 (instead of Day 3) and after transfer of warmed embryos after vitrification (instead of after fresh embryo transfer).

Introduction

Pre-implantation genetic testing for monogenic disorders (PGT-M) and for structural chromosomal aberrations (PGT-SR) are invasive procedures due to the embryo biopsy but offer a recourse for parents to prevent the transmission of a heritable disease to their offspring. While the number of reported PGT cycles increases annually and the number of births reached over 10 000 in 2012 according to the ESHRE PGD Consortium (De Rycke *et al.*, 2017), the health risks for the offspring are still poorly understood. Although couples opting for PGT-M or PGT-SR are not necessarily infertile, they run through all steps associated with ART, including ovarian stimulation and ICSI. Therefore, the medical outcome of children born after ICSI in combination with an embryo biopsy should be closely monitored. Although neonatal outcomes after PGT conception have been found comparable to those after ICSI (Desmyttere *et al.*, 2012; Bay *et al.*, 2016; Hasson *et al.*, 2017), only a few studies have reported on the medical outcome after PGT at older ages. We previously reported a lower BMI in 2-year-old PGT children, while the prevalence of major as well as minor congenital anomalies, hospital admissions and surgical interventions was similar (Desmyttere *et al.*, 2009).

Embryo biopsy in combination with IVF or ICSI is also applied in couples with an infertility background for the purpose of pre-implantation genetic testing for aneuploidy (PGT-A). But parents who opt for PGT-M or for PGT-SR are not necessarily infertile. Although data on outcomes after PGT-A are not directly transferable, Dutch reseachers reported that blood pressure and anthropometry in children conceived after IVF/ICSI with or without PGT-A did not differ at the age 4 and 9 years (Seggers *et al.*, 2013; Kuiper *et al.*, 2017).

In the present single-centre study, we investigate whether ICSI in combination with embryo biopsy at the cleavage stage adversely affects child health by comparing blood pressure readings and body composition in 6-year-old singletons born after PGT-M and PGT-SM with results of case-controlled matched peers born after ICSI.

Material and Methods

Setup and study groups

All singleton, Caucasian, Dutch-speaking children, born after 32 weeks of gestation reaching 6 years of age between May 2011 and June 2017 and born after PGT-M and PGT-SR were eligible. Only Dutch-speaking and Caucasian singletons were considered eligible to overcome linguistic and sociocultural barriers, given the parallel psychomotor testing. Multiples and very prematurely (<32 weeks) born children were not eligible because of possible interference with their development.

For each child in the PGT-M and PGT-SR group, a singleton born after transfer of a fresh ICSI embryo at the blastocyst stage and reaching the age of 6 years between May 2011 and June 2017 was matched as closely as possible for gender, age, maternal educational level (low, medium, high) and finally for birth order. Maternal educational level is used as a proxy for (family) social educational status, which has been shown to be inversely related to child body weight (Murasko, 2009). Matching for birth order was performed given the well-known differences in size at birth and growth during childhood of firstborns vs later-borns (Savage *et al.* 2013). In the case of more than one match, the control child that was invited for participation was randomly selected by the computer. In case the parents refused participation, another child fulfilling the matching criteria was invited.

Given the comprehensive paediatric and neurological follow-up programme for all children born after ART in our centre, all eligible children were previously examined at age 2 months and/or 2 years (Desmyttere *et al.*, 2012). Families were invited for clinical examination at the Centre for Medical Genetics by telephone after they had received a letter explaining the study design. Data on cognitive and psychomotor development were collected in parallel and are reported elsewhere (Winter *et al.*, 2014).

The ovarian stimulation protocol and the ICSI procedure were identical in both groups. All embryo biopsies (aspiration of blastomeres after laser drilling) were carried out on fresh cleavage-stage embryos (Day 3), followed by fresh transfer at the blastocyst stage (Day 5), as previously described (De Vos *et al.*, 2009). Therefore, only children conceived by ICSI after transfer of a fresh embryo on Day 5 were eligible for matching. Only embryo biopsies for the purpose of PGT-M and PGT-SR were considered; PGT-A cycles were excluded.

Measurements

Physical examination

Weight and (standing) height were measured using standard equipment. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Waist circumference and mid-upper arm (MUAC) circumference were measured using a non-stretchable tape. Waist circumference was measured at the narrowest point between the lower costal border and the iliac crest, with the subjects standing. The MUAC was measured midway between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna. Skinfolds were measured at the non-dominant side of the body using a commercial skinfold caliper (Harpenden, British Indicators Ltd., London, UK). Triceps and biceps skinfold thickness were used as a measure of peripheral adiposity, and subscapular and supra-iliac skinfold thickness as a measure of central adiposity. The sum of the four skinfolds was used as a measure of total adiposity and the percentage of body fat was derived from the skinfolds using the Slaughter equation (Slaughter *et al.*, 1988), which is appropriate for both sexes in childhood (Nagy *et al.*, 2014). The central/peripheral (subscapular/triceps) skinfold ratio was used as an index of body fat distribution. Weight, height, BMI, waist circumference and MUAC were converted to age- and sex-adjusted *z*-scores using national reference curves (Roelants *et al.*, 2009).

Solely for the purpose of completeness, we report major congenital malformations defined as a malformation that generally causes functional impairment or requires surgical correction (Bonduelle *et al.*, 2002). Here, we report malformations from birth up to the age of 6 years.

Buccal smears were collected and stored for later epigenetic analysis.

Blood pressure and heart rate were measured twice using an automated electronic device (Spot Vital Signs, Welch Allyn, Inc., New York, NY, USA) on the non-dominant arm when the subject had been seated for at least 5 min. The device has passed international validation protocols for accuracy (Davies *et al.*, 2005; Alpert, 2007). Sex-, height- and age-adjusted *z*-scores of blood pressure readings were calculated according to standards of the National High Blood Pressure Eduction Program Working Group on High Blood Pressure in Children and Adolescents (2004), using height *z*-scores based on the national growth charts (Roelants *et al.*, 2009).

Questionnaires

Parents were asked to complete a questionnaire covering a broad range of parameters related to their and their children's health status. This included information on hospitalizations, chronic medication intake (>3 months), illnesses, treatments (speech, psychological, physiotherapy) and surgical interventions. In addition, the family history and parental height and weight (converted to BMI) and educational level were registered as well as the pregnancy course and early-life factors including breastfeeding and *in utero* exposure to tobacco and alcohol. The parental educational level was coded as low (partially completed education or no qualifications at all), medium (high school degree) or high (degree in higher education).

Informed consent and ethics committee

Written informed consent was obtained from all parents. The study was approved by the Ethics Committee of the UZ Brussel.

Statistical analysis

Descriptive statistics for continuous variables are presented as means with their SD and as the frequency and percentage for categorical variables. A Chi-squared test was used to compare categorical variables and Student's *t*-test to compare continuous variables between the group born after embryo biopsy (PGT-M and PGT-SR) and the ICSI group. A *P* value <0.05 was considered significant. Data analysis was performed using SPSS software version 23 (SPSS Inc, Chicago, IL, USA).

Z-scores for weight, height, BMI and mid-upper arm and waist circumference were calculated from the national growth reference (Roelants *et al.*, 2009). Birthweight *z*-scores were calculated according to Niklasson *et al.* (1991) taking into account gender and gestational age.

Since the study was not powered to comment on congenital malformation rates, no statistical analyis was performed for this outcome.

Multiple linear regression analysis was used to investigate differences in body measurements and blood pressure between the two study groups, adjusted for covariates. Results are expressed as unstandardized regression coefficients (B) with a 95% CI. Covariates known to affect body composition and covariates that differed among groups were included in the final model: these include current characteristics (age), neonatal characteristics (birthweight, birth order) and parental characteristics (maternal smoking during pregnancy, BMI of the mother and of the father). For the analysis of blood pressure, BMI was included in the model as an additional covariate.

Subgroup multiple linear regression analysis was performed comparing anthropometrics and blood pressure readings in couples with an infertility factor whether related to their genetic problem or found incidentally.

The required sample size was estimated at 90 PGT-M and PGT-SR children and 90 ICSI children in order to detect a difference in systolic and diastolic blood pressure and waist circumference of 0.4–0.5 times the SD with a power of 80% and a test probability of 5%. This corresponds to a

difference in either systolic or diastolic blood pressure of 4–5 mmHg, assuming an SD of 10 mmHg (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004) or a difference of 1.6–2 cm for waist circumference.

Results

Participation

From the 124 eligible PGT-M and PGT-SR families, 110 could be reached of whom 23 refused and 87 (87/110 = 79%) participated. 'No time' or 'too long distance to the hospital' were the main reasons for refusing participation. Non-participating PGT-M and PGT-SR children (n = 23) did not differ from the participating PGT children in terms of gender, being firstborn, gestational age, birthweight or maternal educational level (data not shown).

From the 140 eligible families with an ICSI child matching a child born after embryo biopsy, 17 could not be reached and 36 refused participation. The reasons for refusal were as follows: too long distance to the hospital, no time, practical problems related to holiday/work to fix a date for the visit. None of the families refused because of (adverse) medical condition of the eligible child. Finally, 87 ICSI children participated (87/123 = 70.7%).

Characteristics of the study population

Neonatal characteristics, including birthweight, birthweight z-score and gestational age were comparable between the two groups (Table I). The groups did also not differ in terms of parental characteristics: maternal weight gain, smoking and alcohol consumption during pregnancy, maternal age, maternal pre-pregnancy and current BMI as well as current paternal BMI were comparable between the groups.

PGT was in 17 cases performed because of chromosomal aberrations (PGT-SR), in 51 cases for monogenic disorders (40 autosomal dominant, 11 autosomal recessive) (PGT-M) and in 19 cases for X-linked disorders (PGT-M).

In 33 (38%) couples opting for PGT-M and PGT-SR, there was an infertility problem. This was in 17 cases related to the genetic disorder requiring PGT-SR and was found incidentally in 16 couples.

In the comparison group, ICSI was performed for male factor infertility in 48 (55%) of the cases, for female factor infertility in 15 of the cases and for combined infertility in 14 cases. The remaining 10 cases were considered as idiopathic infertility.

For information only, major congenital malformations were detected in seven PGT-M and PGT-SR children and in nine children born after ICSI. The following malformations were noted: in PGT-M and PGT-SR children—extensive congenital naevus, inguinal hernia (n = 2) requiring surgery, spermatocele, cryptorchidism, lymphangioma, cleft palate and in children born after ICSI—extensive congenital naevus, inguinal hernia requiring surgery, spermatocele, cryptorchidism, strabism (n = 3) requiring surgery, congenital hip dysplasia, sublingual thyroid.

Body composition after PGT-M and PGT-SR and ICSI

Anthropometric measurements, including z-scores of weight, height and BMI were comparable between the two groups (Table II). Also, circumferential measurements (waist and mid-upper arm) were comparable between PGT-M and PGT-SR vs ICSI children. Furthermore, indices of peripheral and central adiposity were comparable between the two groups. The body fat mass, calculated according to the Slaughter equation, did not differ between children born after PGT-M and PGT-SR or ICSI.

Also in the adjusted analysis, taking into account current, neonatal and parental characteristics, no differences were found between z-scores of BMI, mid-upper arm and waist circumference and calculated body fat mass in PGT-M and PGT-SR vs ICSI controls.

Resting blood pressure after PGT-M and PGT-SR and ICSI

Mean systolic and diastolic blood pressure z-scores were comparable between the two groups (Table II). Also, systolic and diastolic blood pressure z-scores did not differ between the two groups when adjusted for current, neonatal and parental characteristics.

Results from questionnaires

Up to the age of 6 years, 19 children born after PGT-M and PGT-SR had been hospitalized at least once, mainly for infectious disease (n = 15), but also for treatment of an extensive lymphangioma (n = 1), seizures (n = 1), constipation (n = 1) and exploration for a nephrologic problem (n = 1). In total, 24 ICSI children were at least once hospitalized, also mainly for infectious disease (n = 19). The rate of hospitalization did not differ between the two groups (P = 0.48). The proportion of chronic medication use (PGT-M and PGT-SR: 7/87; ICSI:6/87) and the proportion of children requiring (temporary or ongoing) additional therapy such as speech therapy, physiotherapy or psychological help (PGT-M and PGT-SR: 13/87; ICSI: 16/87) were not different between children born after embryo biopsy and after ICSI (P = 1.0 and P = 0.68, respectively). Surgical intervention was not more often required in children born after embryo biopsy (24/87) than for children born after ICSI (38/87) (P = 0.62). Surgical intervention was required mostly (PGT-M and PGT-SR: 16/24; ICSI: 18/28) for minor indications such as adenotonsillectomy and/or placement of grommets. Other indications were as follows: in the PGT-M and PGT-SR group: cryptorchidism, spermatocele, inguinal hernia, naevus, circumcision, cleft palate, lymphangioma and in the ICSI group: cryptorchidism, spermatocele, inguinal hernia, naevus, circumcision, strabism, burn wound, spleen rupture.

Subgroup analysis in children conceived by PGT-M and PGT-SR from parents with an infertility factor

Z-scores of BMI, mid-upper arm and waist circumference, the sums of peripheral and central skinfold thicknesses, and z-scores of systolic and diastolic blood pressure in 33 children born after PGT-M and PGT-SR from parents with an infertility component were also comparable with the results in all ICSI children, also after adjustment for confounders (data not shown).

Discussion

This study investigates the medical outcome of the largest single-centre cohort of singletons born after PGT for monogenic disorders

Table I Neonatal and parental characteristics of the population in a matched cohort study of body composition and blood pressure in 6-year-old singletons born after PGT.

	PGT-M and PGT-SR *N* = 87	ICSI *N* = 87	*P***
Neonatal characteristics*			
Child gender male (*n*)	39 (45)	39 (45)	
Firstborn (*n*)	49 (56)	56 (64)	0.3
Birthweight (g)	3267 ± 616	3357 ± 475	0.3
Birthweight *z*-score	0.02 ± 0.96	0.05 ± 0.92	0.8
Gestational age in weeks	38.6 ± 2.2	39.0 ± 1.5	0.1
Prematurity <37 weeks (*n*)	10 (11)	8 (9)	0.8
Parental characteristics*			
Maternal smoking in pregnancy (*n*)	2 (2)	6 (7)	0.3
Maternal alcohol consumption in pregnancy (*n*)	2 (2)	4 (5)	0.4
Weight gain during pregnancy (kg)	13.1 ± 5.2	14.2 ± 5.6	0.2
Maternal age (years)	30.5 ± 3.3	31.1 ± 3.5	0.2
Maternal educational level: higher level (*n*)	57 (65)	56 (64)	0.9
Maternal pre-pregnancy BMI (kg/m^2)	23.9 ± 4.3	23.4 ± 4.8	0.5
Current maternal BMI (kg/m^2)	23.8 ± 3.8	23.6 ± 4.1	0.8
Current paternal BMI (kg/m^2)	25.5 ± 3.4	25.1 ± 3.9	0.8
PGT for monogenetic disease (*n*)	51 (58.5)		
PGT for chromosomal aberration (*n*)	17 (19.5)		
PGT for X-linked disease (*n*)	19 (22)		

*Continuous data: mean ± SD and categorical data: number (%).
**Chi-square test or Student's *t*-test as appropriate.
PGT-M, pre-implantation genetic testing for monogenic disease; PGT-SR, PGT for structural chromosomal aberrations.

and chromosomal structural aberrations, having reached the age of 6 years. Compared to matched peers born after ICSI without embryo biopsy, children born after PGT-M and PGT-SR showed no differences in body composition measurement and blood pressure.

Comparing our data to literature data is difficult since follow-up data of children conceived by PGT beyond infancy are scarce. Data on obstetric and neonatal outcomes were recently analysed by Hasson *et al.* (2017) and no adverse effect of PGT was found. We previously reported that, at 2 years of age, the mean BMI *z*-score although within normal limits tended to be lower in children born after PGT-M, PGT-SR or PGT-A, while the prevalence of major as well as minor congenital anomalies, hospital admissions and surgical interventions was similar (Desmyttere *et al.*, 2009). In this study, where only singletons born after PGT-M and PGT-SR but not after PGT-A were included, we did not find any difference in BMI at the age of 6 years. Only one other research group described anthropometric and blood pressure measurements at childhood ages in children born after embryo biopsy in a smaller study: at 4 and 9 years of age they found no differences between 43 children (29 singletons, 14 twins) born after IVF/ICSI with PGT-A and 56 children born after IVF/ICSI without PGT-A (Seggers *et al.*, 2013; Kuiper *et al.*, 2017). However, although embryo biopsy was also performed at Day 3 in the latter studies, outcomes in PGT-A children may not be extrapolated to PGT-M and PGT-SR children given the dissimilar indications and parental reproductive background.

Although BMI is the most frequently used parameter for screening of excess adiposity (Sarría *et al.*, 2001) and cardiometabolic morbidity (Adaki *et al.*, 2013), its limitations especially in growing children are well known (Wells, 2000). Indeed a high variability in body fat for a given BMI in children is not uncommon (Wells *et al.*, 2006). This limitation can be partly addressed by inclusion of other anthropometric parameters and indices. We therefore also reported skinfold thickness and waist and mid-upper arm circumferences. Although these anthropometric measurements provide crucial information regarding body composition, the distribution of body fat and the prediction of cardiometabolic risk in children (Rose, 2005), measurements like dual-X-ray absoprtiometry measurements are needed to discriminate between fat and lean body mass. Furthermore, interpretation and comparison of data on body composition at the individual and population level is often impeded by the lack of appropriate reference data. We, unlike others, have expressed anthropometric and blood pressure results as *z*-scores, thereby effectively taking into account gender and age.

However, although no differences were found between the PGT-M and PGT-SR and ICSI offspring, the reported *z*-scores for blood pressure are significantly higher than 0 in both groups. This indicates that the blood pressure is not negatively affected by the embryo biopsy, but it remains currently unclear whether there is an overall effect of ICSI as our study did not include a control group of spontaneously conceived peers, and also because the literature on this topic is not univocal (Guo *et al.*, 2017).

Our findings are robust even after adjustments for covariates that are generally known to affect body composition. The matching of

Table II Anthropometrics and blood pressure results of the PGT-M and PGT-SR and ICSI children.

	PGT-M and PGT-SR *N* = 87	ICSI *N* = 87	*P***	Mean difference; 95% CI	Adjusted* mean difference; 95% CI
Age (years)	5.5 ± 0.4	5.6 ± 0.4	0.2		
Head circumference (cm)	51.1 ± 1.4	51.4 ± 1.5	0.3		
Head circumference *z*-score	0.18 ± 0.94	0.31 ± 0.97	0.4	−0.13; −0.41, 0.16	−0.18; −0.45, 0.09
Weight (kg)	19.9 ± 3.0	19.9 ± 2.7	0.8		
Weight *z*-score	−0.04 ± 1.0	−0.13 ± 1.0	0.5	0.09; −0.21, 0.39	0.06; −0.22, 0.35
Height in cm	114.0 ± 5.7	114.2 ± 5.3	0.9		
Height *z*-score	0.18 ± 0.99	0.08 ± 1.1	0.5	0.09; −0.22, 0.41	0.04; −0.27, 0.34
BMI in kg/m^2	15.3 ± 1.3	15.2 ± 1.4	0.7		
BMI *z*-score	−0.23 ± 1.0	−0.29 ± 0.97	0.7	0.05; −0.24, 0.35	0.05; −0.24, 0.34
Blood pressure					
Systolic in mmHg	100.5 ± 5.9	101.2 ± 8.2	0.5		
Systolic *z*-score	0.48 ± 0.55	0.55 ± 0.75	0.4	−0.08; −0.28, 0.12	−0.07; −0.28, 0.13
Diastolic in mmHg	63.7 ± 6.6	63.5 ± 7.7	0.9		
Diastolic *z*-score	0.71 ± 0.60	0.69 ± 0.66	0.8	0.02; −0.17, 0.20	0.02; −0.17, 0.22
Sum of peripheral skinfolds (mm)	14.7 ± 4.5	15.5 ± 4.3	0.2	−0.9; −2.3, 0.4	−1.2; −2.6, 0.2
Sum of central skinfolds (mm)	11.6 ± 3.8	11.5 ± 3.7	0.9	0.1; −1.1, 1.2	−0.2; −1.3, 0.9
Total sum of skinfolds (mm)	26.3 ± 7.3	26.9 ± 7.5	0.4	−0.9; −3.3, 1.5	−1.4; −3.8, 0.9
Subscapular/triceps skinfold ratio	0.6 ± 0.1	0.6 ± 0.2	0.9		
Total body fat (%)°	13.7 ± 3.3	13.9 ± 3.7	0.6	−0.2; −1.4, 0.9	−0.4; −1.5, 0.7
Waist circumference (cm)	53.2 ± 4.0	52.6 ± 3.7	0.4		
Waist circumference *z*-score	0.27 ± 0.95	0.11 ± 1.1	0.3	0.16; −0.14, 0.47	0.17; −0.13, 0.48
Mid-upper arm circumference (cm)	17.6 ± 1.5	17.5 ± 1.4	0.8		
Mid-upper arm circumference z-score	0.17 ± 0.80	0.11 ± 0.85	0.6	0.06; −0.18,0.31	0.02; −0.23, 0.26

Continuous data: mean ± SD.
°According to Slaughter equation.
*Adjusted for current characteristics (age), neonatal characteristics (birthweight, birth order) and parental characteristics (maternal smoking during pregnancy, BMI of the mother and of the father).
**Student' *t*-test.

PGT-M and PGT-SR singletons according to maternal, pregnancy and sociodemographical characteristics to peers born after ICSI without embryo biopsy is a major strength of this study, since these variables can influence prenatal as well as postnatal growth. Consequently, the only difference in procedure between the two groups of this single-centre study is the embryo biopsy since all transfers comprised fresh embryo transfers and all were carried out at Day 5. However, since we are aware that a proportion of the couples requiring PGT-M and PGT-SR also face an infertility problem, we performed a subgroup analysis comparing outcomes in singletons born after PGT-M and PGT-SR from parents with a concomitant infertility problem vs ICSI children in order to unravel the effect of the invasive procedure (biopsy) vs the parental background (infertility). Although we recognize this group is rather small, but still somewhat larger than the number of singletons described by Kuiper *et al.* (2017), we did not find differences in anthropometrics and blood pressure measurements between children born after PGT-M and PGT-SR from parents with an infertility component and ICSI children.

Overall, with a sample of 87 PGT-SR and PGT-M singleton children, which is the largest cohort of singletons born after embryo biopsy, we were able to detect moderate differences in anthropometrics and blood pressure, but the detection of small differences requires even larger sample sizes. Despite this observation, no clinically relevant differences were found between children born with/without embryo biopsy. Also, the finding of comparable hospitalization and surgical intervention rates adds to this reassurance.

In conclusion, in this single-centre matched-pair cohort study of 5–6-year-old children born after PGT-M and PGT-SR and ICSI, no differences in anthropometrics and blood pressure measurements were found. However, more and long-term follow-up studies are indicated as there are currently no data available for PGT children born after trophectoderm biopsy and/or transfer of warmed embryos after vitrification.

Acknowledgements

We are grateful to all parents who participated in the study. We are extremely thankful to our study nurses Andrea Buysse and Leen Ausloos for contacting the families. Special thanks also go to Berthold Aman and to Walter Meul.

Authors' roles

The study was designed by S.D., C.W., H.T., I.L., M.D.R. and M.B. Data were collected by S.K., F.D.S. and were analysed by F.B. and M.R. All co-authors interpreted the data. F.B. wrote the paper, and it was finalized by all co-authors. All co-authors approved the definitive version of the manuscript.

Funding

This study was supported by Methusalem grants and by grants from Wetenschappelijk Fonds Willy Gepts; all issued by the Vrije Universiteit Brussel (VUB).

Conflict of interest

All co-authors, except MB declared no conflict of interest. MB. has received consultancy fees from MSD, Serono Symposia and Merck. The Universitair Ziekenhuis Brussel (UZ Brussel) and the Centre for Medical Genetics have received several educational grants from IBSA, Ferring, Organon, Shering-Plough, Merck for establishing the database for follow-up research and organizing the data collection.

References

Adaki ACR, Tinico ALA, Mendes EL, Junior RA, Hills AP, Amorim PRS. Anthropometry and physical activity level in the prediction of metabolic syndrome in children. *Public Health Nutr* 2013;**24**:1–8.

Alpert B. Validation of the Welch Allyn Spot Vital Signs blood pressure device according to the ANSI/AAMI SP10: 2002. Accuracy and cost-efficiency successfully combined. *Blood Press Monit* 2007;**12**:345–347.

Bay B, Ingerslev HJ, Lemmen JG, Degn B, Rasmussen IA, Kesmodel US. Preimplantation genetic diagnosis: a national multicenter obstetric and neonatal follow-up study. *Fertil Steril* 2016;**106**:1363–1369.e1. doi:10.1016/j.fertnstert.2016.07.1092.

Bonduelle M, Liebaers I, Deketelaere V, Derde MP, Camus M, Devroey P, Van Steirteghem A. Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999). *Hum Reprod* 2002;**17**:671–694.

Davies P, Dennis J, Railton R. Evaluation of the A&D UA-767 and Welch Allyn Spot Vital Signs noninvasive blood pressure monitors using a blood pressure simulator. *J Hum Hypertens* 2005;**19**:197–203.

De Rycke M, Goossens V, Kokkali G, Meijer-Hoogeveen M, Coonen E, Moutou C. ESHRE PGD Consortium data collection XIV-XV: cycles from January 2011 to December 2012 with pregnancy follow-up to October 2013. *Hum Reprod* 2017;**32**:1974–1994. doi:10.1093/humrep/dex265.

De Vos A, Staessen C, De Rycke M, Verpoest W, Haentjens P, Devroey P, Liebaers I, Van de Velde H. Impact of cleavage-stage embryo biopsy in view of PGD on human blastocyst implantation: a prospective cohort of single embryo transfers. *Hum Reprod* 2009;**24**:2988–2996. doi:10.1093/humrep/dep251.

Desmyttere S, Bonduelle M, Nekkebroeck J, Roelants M, Liebaers I, De Schepper J. Growth and health outcome of 102 2-year-old children conceived after preimplantation genetic diagnosis or screening. *Early Hum Dev* 2009;**85**:755–759. doi:10.1016/j.earlhumdev.2009.10.003.

Desmyttere S, De Rycke M, Staessen C, Liebaers I, De Schrijver F, Verpoest W, Haentjens P, Bonduelle M. Neonatal follow-up of 995 consecutively born children after embryo biopsy for PGD. *Hum Reprod* 2012;**27**:288–293. doi:10.1093/humrep/der360.

Guo XY, Liu XM, Jin L, Wang TT, Ullah K, Sheng JZ, Huang HF. Cardiovascular and metabolic profiles of offspring conceived by assisted reproductive technologies: a systematic review and meta-analysis. *Fertil Steril* 2017;**107**:622–631.e5. doi:10.1016/j.fertnstert.2016.12.007.

Hasson J, Limoni D, Malcov M, Frumkin T, Amir H, Shavit T, Bay B, Many A, Almog B. Obstetric and neonatal outcomes of pregnancies conceived after preimplantation genetic diagnosis: cohort study and meta-analysis. *Reprod Biomed Online* 2017;**35**:208–218. doi:10.1016/j.rbmo.2017.05.003.

Kuiper D, Bennema A, la Bastide-van Gemert S, Seggers J, Schendelaar P, Mastenbroek S, Hoek A, Heineman MJ, Roseboom TJ, Kok JH *et al*. Developmental outcome of 9-year-old children born after PGS: follow-up of a randomized trial. *Hum Reprod* 2017;**10**:1–9. doi:10.1093/humrep/dex337.

Murasko JE. Socioeconomic status, height, and obesity in children. *Econ Hum Biol* 2009;**7**:376–386. doi:10.1016/j.ehb.2009.04.004.

Nagy P, Kovacs E, Moreno LA, Veidebaum T, Tornaritis M, Kourides Y, Siani A, Lauria F, Sioen I, Claessens M *et al*. Percentile reference values for anthropometric body composition indices in European children from the IDEFICS study. *Int J Obes (Lond)* 2014;**38**:S15–S25. doi: 10.1038/ijo.2014.131.

National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* 2004;**114**:555–576.

Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977–1981). *Acta Paediatr Scand* 1991;**80**:756–762.

Roelants M, Hauspie R, Hoppenbrouwers K. References for growth and pubertal development from birth to 21 years in Flanders, Belgium. *Ann Hum Biol* 2009;**36**:680–694. doi:10.3109/03014460903049074.

Rose G. Incubation period of coronary heart disease. *Int J Epidemiol* 2005;**34**:242–244.

Sarría A, Moreno LA, García-Llop LA, Fleta J, Bueno M. Body mass index, triceps skinfold and waist circumference in screening for adiposity in male children and adolescents. *Acta Paediatr* 2001;**90**:387–392.

Savage T, Derraik JG, Miles HL, Mouat F, Cutfield WS, Hofman PL. Birth order progressively affects childhood height. *Clin Endocrinol (Oxf)* 2013;**79**:379–385. doi:10.1111/cen.12156.

Seggers J, Haadsma ML, Bastide-van Gemert Sl, Heineman MJ, Kok JH, Middelburg KJ, Roseboom TJ, Schendelaar P, Van den Heuvel ER, Hadders-Algra M *et al*. Blood pressure and anthropometrics of 4-y-old children born after preimplantation genetic screening: follow-up of a unique, moderately sized, randomized controlled trial. *Pediatr Res* 2013;**74**:606–614. doi:10.1038/pr.2013.137.

Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, van Loan MD, Bemben DA. Skinfold equations for estimation of body fatness in children and youths. *Hum Biol* 1988;**60**:709–723.

Wells JCK. A Hattori chart analysis of body mass index in infants and children. *Int J Obes* 2000;**24**:325–329.

Wells JCK, Fewtrell MS, Williams JE, Haroun D, Lawson MS, Cole TJ. Body composition in normal weight, overweight and obese children: matched casecontrol analysis of total and regional tissue masses, and body composition trends in relation to relative weight. *Int J Obes* 2006;**30**:1506–1513.

Winter C, Van Acker F, Bonduelle M, Desmyttere S, De Schrijver F, Nekkebroeck J. Cognitive and psychomotor development of 5- to 6-year-old singletons born after PGD: a prospective case-controlled matched study. *Hum Reprod* 2014;**29**:1968–1977. doi:10.1093/humrep/deu165.