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Blood pressure in ICSI-conceived adolescents

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STUDY QUESTION: Do young adolescents conceived by ICSI display a higher blood pressure than spontaneously conceived (SC) adolescents?

SUMMARY ANSWER: In our study, 14-year-old male and female ICSI teenagers were not found to have increased blood pressure at rest.

WHAT IS KNOWN AND WHAT THIS PAPER ADDS: Only limited data are available regarding the cardiovascular risk of children born after assisted conception and up till now, no data on the cardiovascular health in pubertal children conceived by ICSI have been published. In this study, resting blood pressure and blood pressure response to a psychological stressor were measured in a cohort of 14-year-old teenagers conceived by ICSI and compared the results with those of a group of SC peers.

DESIGN: In this cross-sectional study, resting blood pressure measurements were available from 217 singleton ICSI children (116 boys, 101 girls) and 223 singleton control children born after spontaneous conception (115 boys, 108 girls). Continuous blood pressure measurements, performed during a psychological stress test, were available for only 67 ICSI and 38 SC children.

PARTICIPANTS AND SETTING: The study group comprised adolescents conceived by ICSI predominantly because of male factor infertility and they were part of a previously published cohort followed since birth; controls were a cross-sectional sample of peers born to fertile parents and recruited from comparable schools as those attended by the ICSI teenagers. Response rates were 56% (tested/reached) in the ICSI group and 50% (agreed/eligible) in the SC group, but information regarding health could be obtained in 63 and 72% of the ICSI and SC children, respectively.

MAIN RESULTS AND THE ROLE OF CHANCE: ICSI girls had a comparable resting systolic (109 $\pm$ 9 mmHg) and diastolic (64 $\pm$ 6 mmHg) blood pressure in comparison with girls in the SC group (111 $\pm$ 9 mmHg, $P = 0.2$ and 66 $\pm$ 7 mmHg, $P = 0.05$), even after adjustment for age and height. After adjustment for current body characteristics, early life and parental background factors, systolic and diastolic blood pressure remained comparable in both groups. In ICSI boys, a slightly lower systolic (113 $\pm$ 10 mmHg), but comparable diastolic (64 $\pm$ 6 mmHg) resting blood pressure was found in comparison with the SC group (116 $\pm$ 9 mmHg; $P = 0.04$ and 65 $\pm$ 5 mmHg; $P = 0.1$). After adjustment for height and age, systolic and diastolic blood pressure were comparable in both groups ($P = 0.7$ and $P = 0.6$). After correction for current body characteristics, early life and parental factors, ICSI and SC boys still had comparable systolic (difference in ICSI versus SC: −1.1 mmHg; 95% CI: −3.8–1.6; $P = 0.4$) and diastolic (difference in ICSI versus SC: −1.2 mmHg; 95% CI: −3.2–0.7; $P = 0.2$) blood pressure measurements. In the small subsample of girls and boys with continuous blood pressure readings, the systolic and diastolic blood pressure response to the stress test was not significantly different between the ICSI and SC groups even after taking into account the baseline values.

BIAS, CONFOUNDING AND OTHER REASONS FOR CAUTION: Despite the rather low response rate in the ICSI group and the fact that no information on current health status could be obtained from more than a quarter of the eligible comparison group, the non-participating analysis in the ICSI as well in the SC group did not reveal differences between participating and non-participating children regarding clinical characteristics. The negative results for the sub-analysis on blood pressure response to stress should be interpreted with caution, because these data were available for only a small number of children, and the analysis may be underpowered. This result can only rule out a large effect on blood pressure responsiveness to a psychological stressor. Although our sample size appears to be appropriate, our results need confirmation by others and in larger cohorts when more data become available.

GENERALIZABILITY TO OTHER POPULATIONS: Our results are the first described ever in ICSI offspring, born to parents suffering from predominantly male factor infertility.

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Key words: ICSI / blood pressure / pubertal / cardiovascular / hypertension

Introduction

Since IVF techniques have been widely available for the treatment of infertility, there has been a concern about possible long-term consequences of embryo manipulation on the development and health of *in vitro* conceived individuals.

Evidence from studies in humans and animals suggests that the periconceptional period, both *in vivo* and *in vitro* is particularly important for later cardiometabolic function, including blood pressure (Watkins and Fleming, 2009). Dietary manipulations during pregnancy in a number of mammalian species have been associated with high blood pressure in the offspring (Kwong *et al*., 2000; Gopalakrishnan *et al*., 2004). Studies in mice have indicated that *in vitro* embryo culture leads to changes in post-natal phenotype in the offspring including raised systolic blood pressure (Lonergan *et al*., 2003; Watkins *et al*., 2007). In humans, the periconceptional maternal diet has been suggested to influence the onset of coronary artery disease, although no increases in hypertension were observed in individuals prenatally exposed to the Dutch famine (Roseboom *et al*., 2000; Painter *et al*., 2006a). Exaggerated blood pressure responses to psychological stressors have been implicated as risk factors for the development of cardiovascular disease, independently from resting blood pressure (Barnett *et al*., 1997; Jennings *et al*., 2004). In different species, including humans, adverse events during prenatal life have been found to cause an increased blood pressure response to psychological stressors (Ward *et al*., 2004; Painter *et al*., 2006a).

To date, studies concerning the effects of IVF in humans on cardiovascular risk are limited. We have previously reported higher blood pressure in 8-year-old children born after ICSI compared with spontaneously conceived (SC) peers (Belva *et al*., 2007). Ceelen *et al*. (2008) showed that IVF children aged 8–18 years have a higher systolic and diastolic blood pressure which could not be explained by current body size, early life factors or parental characteristics. Recently, Sakka *et al*. (2010) confirmed these findings in children aged 4–14 years born after IVF. However, the mechanisms responsible for the increased blood pressure are not well understood. Scherrer *et al*. (2012) recently showed that, even in the absence of alterations in resting blood pressure, children conceived by IVF/ICSI had increased pulse wave velocity and markers of vascular dysfunction, indicating that multiple factors contributing to cardiovascular risk might be affected by IVF/ICSI. Up to now, the blood pressure reaction to a psychological stressor, as a separate risk factor of cardiovascular disease, has not been studied in offspring after assisted reproductive technology (ART).

In the present study, we have investigated if at adolescence, ICSI conception is associated with a higher resting and stress-induced blood pressure in the very first cohort of children conceived by ICSI in comparison with a group of SC peers.

Materials and Methods

Set-up and study population

This study is part of a prospective follow-up study on cardiometabolic risk in the very first cohort of children born after transfer of fresh embryos obtained by ICSI using ejaculated and non-ejaculated sperm.

ICSI and SC children were eligible for inclusion if they were Caucasian, singleton and born after at least 32 weeks of gestation. Children born small-for-gestational age (SGA) were not excluded.

Results were described at the age of 14 years since the worldwide oldest ICSI cohort reached the age of 14 years during the study period and because all children in Belgium have a scheduled medical visit at this age, which facilitated recruitment of SC controls.

Of the eligible cohort of 501 ICSI children, 217 (116 boys, 101 girls) reaching the age of 14 years between January 2008 and March 2011, were examined at the Center for Medical Genetics after an invitation by phone; 116 could not be reached and 168 refused to participate, yielding a participation rate of 56% (tested/reached). ICSI was predominantly performed because of male factor infertility (86%); other indications were female factor infertility (6%), combined infertility (5%) and unexplained infertility (3%). Birthweight (3219 ± 574 versus 3280 ± 496 g; $P = 0.2$), gestational age (38.6 ± 1.5 weeks versus 38.7 ± 1.3 weeks; $P = 0.3$), prematurity rate (8 versus 5%; $P = 0.3$) and maternal age (31.3 ± 3.7 versus 32.0 ± 4.6 years; $P = 0.1$) were comparable between the non-participants (not reached + refusal) and the participants. The proportion of higher educated mothers was lower (53 versus 66%; $P = 0.01$) in the non-participants in comparison with the participants. Families who refused to participate were asked minimal information on the health of their child and this information was obtained from 105 (54 males; 51 females) children (63%). ICSI children who refused participation did not differ from participating ICSI peers regarding male gender (51 versus 53%, $P = 0.8$), attending general education (70 versus 74%, $P = 0.5$) or current body weight (females: 50.9 ± 7.8 kg versus 53.1 ± 8.7 kg, $P = 0.1$; males: 52.1 ± 9.7 versus 53.0 ± 12.1 kg, $P = 0.6$).

A comparison group of 223 children born after spontaneous conception (SC) and without the use of hormonal stimulation was recruited by letter in comparable types of schools as those attended by the ICSI children. Out of 553 invited children, initially 327 agreed to participate, of whom 278 children met the inclusion criteria and 223 children (115 boys, 108 girls) were examined; 83 refused and 143 did not respond to our invitation. Response rates are described in detail elsewhere (Belva *et al*., 2012). Children who refused did not differ from their participating peers with respect to the proportion of males (43 versus 51%), birthweight (3403 ± 500 versus 3436 ± 481 g, $P = 0.6$), gestational age (39.1 ± 1.5 versus 39.4 ± 1.5 weeks, $P = 0.2$), attending general education (73 versus 81%, $P = 0.2$), current body weight (females: 52.8 ± 7.6 versus 51.6 ± 7.2 kg, $P = 0.4$; males: 54.6 ± 10.9 versus 56.1 ± 11.5 kg, $P = 0.5$), current height (females: 165.1 ± 5.8 versus 163.3 ± 6.8 cm, $P = 0.1$; males: 168.9 ± 8.6 versus 170.3 ± 7.8 cm, $P = 0.4$) and maternal age (29.3 ± 3.9 versus 28.5 ± 4.4 years, $P = 0.2$). As in the ICSI group, non-participating mothers were however less likely to be highly educated (50 versus 68%, $P = 0.01$).

To estimate if non-participation affected our results, we pooled available data on weight of ICSI and SC teenagers who refused participation, with that of the participants, and reanalyzed weight according to population. Pooling participants and non-participants did not change the reported differences in weight between the ICSI and SC population, neither in females or in males (Belva *et al.*, 2012).

Data from a physical examination were supplemented with data from a parental questionnaire on anthropometric, medical and socio-economic indicators including the child's personal and family history. Regarding the parents, maternal and paternal age, current body mass index (BMI), educational level and medical antecedents were recorded. Educational level, as a proxy of socio-economic status was classified as low (secondary school or lower) or high (Bachelor degree or higher). Information on pregnancy-induced disorders (hypertension, pre-eclampsia, gestational diabetes, thyroid) were collected by means of self report. Parental risk for cardiometabolic disease was defined as either one or both of the parents suffering, at the time of the visit, from one or more of the following disorders/diseases requiring medical and or surgical treatment: hypertension, diabetes mellitus, hypercholesterolemia or cardiovascular disease such as bypass or stenting. Regarding the children, information on birth, health and medication, school type (general or vocational education) and sports participation (hours/week) was obtained.

All parents gave written informed consent. The study was approved by the ethics committee of the UZ Brussel.

Measurements

All participants were examined by one paediatrician (F.B.), who was not blinded to the mode of conception due to the recruitment strategy. Anthropometric data on body weight and height were collected with standard equipment to the nearest 0.1 kg and 0.1 cm. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Puberty was evaluated using Tanner staging (Tanner and Whitehouse, 1976). Although we recorded genital and breast development as well as axillary and pubic hair development, we used the Tanner score of genital development in males and breast development in females as parameters in our analysis. Height, weight and BMI were converted to standard deviation scores (SDS) using national reference curves (Roelants *et al.*, 2009), and thus corrected for age and gender. Birthweight was also expressed as SDS in order to correct for gestational age and gender.

Resting blood pressure was the arithmetic mean of two measurements taken from the non-dominant arm and taken when the child had been seated for at least 5 min. Intra-person variation was 1.9 ± 6.7 mmHg for systolic and 1.8 ± 5.5 mmHg for diastolic blood pressure in females and 2.5 ± 7.1 mmHg for systolic and 1.7 ± 5.7 mmHg for diastolic blood pressure in males. All measurements were performed using the Spot Vital Signs (Wellch Allyn, Inc.), an automated electronic device, which automatically inflates and deflates the upper arm cuff. The device has passed international validation protocols for accuracy (Davies *et al.*, 2005; Alpert, 2007). Cuff sizes were chosen according to the recommendations by Pickering *et al.* (2005) based on arm circumferences.

To assess blood pressure in response to a mild psychological stressor, blood pressure was measured continuously during a baseline line period and during the psychological stress test using a Finometer (Finapres Medical Systems, Amsterdam, The Netherlands), which has been shown a reliable device for tracking blood pressure both in adults and in children (Tanaka *et al.*, 1994). These measurements were only taken from a subgroup (ICSI: $n = 67$; SC: $n = 38$) examined between July 2010 and March 2011 due to the availability of the device. In all children, in both the ICSI and SC groups examined from July 2010 onwards, finometer measurements were performed. The stress protocol started with a 2-min baseline period, followed by a computerized Stroop (colour-word conflict) test which took 5 min. The Stroop test is a neuropsychological test often used and validated in children for the assessment of executive functioning (Robinson *et al.*, 2009; Ikeda *et al.*, 2012).

The Stroop test was followed by a 2-min recovery period. Firstly, baseline stress test values which represent the mean of continuous measurements from the baseline period in the stress protocol were obtained. Secondly, we calculated the stress response by subtracting the mean baseline value from the mean value during the stress test, for systolic blood pressure, diastolic blood pressure and heart rate.

ICSI as well as SC children were randomly examined in the morning or in the afternoon. Study protocols were identical for ICSI and SC children. ICSI children were examined at the department of Medical Genetics and SC children were examined outside the hospital.

Statistical analysis

Continuous data are presented as means and standard deviations (SDs), and were compared among groups by a Student's *t*-test. Categorical data are presented as the number of children and the percentage in a certain category. A χ^2 test was used to assess the statistical significance of observed differences between groups and the Pearson correlation coefficient (*r*) was provided for correlations.

Longitudinal data (height SDS and weight SDS) were available from the ICSI children (females: $n = 101$; males: $n =$) at the following ages: birth (females: $n = 101$; males: $n = 116$), 3 months (females: $n = 96$; males: $n = 104$) and 1 year (females: $n = 54$; males: $n = 51$). We calculated the differences in weight SDS between two time points (weight gain): Δ weight SDS birth–3 months (weight gain at early infancy) and Δ weight SDS 3 months–1 years (weight gain at late infancy). Further on, weight gain at every period was stratified into weight gain <percentile 50 (slow weight gain) and weight gain >percentile 50 (rapid weight gain). Finally, systolic and diastolic blood pressure was compared for ICSI children with slow and fast weight gain for the two periods (early and late infancy).

Due to the well-known sexual dimorphism of blood pressure, particularly at puberty, all results are stratified according to gender (Shankar *et al.*, 2005). The association between mode of conception and blood pressure and between mode of conception and blood pressure response after stress was assessed with multiple linear regression, with or without adjustment for potential confounders. Firstly, we examined associations between blood pressure and current characteristics (age, weight, height, pubertal stage). Secondly, we examined the influence of early life factors (gestational age, birthweight and parity) and lastly, we examined the influence of parental characteristics (maternal age, maternal educational level, maternal BMI, pregnancy-induced hypertension and parental risk estimates for cardiometabolic disease). The influences of early life factors and parental characteristics were explored after prior adjustment for age and height because of differences in baseline characteristics. Results are expressed as unstandardized regression coefficients (*B*) with a 95% confidence interval (95% CI), which is an estimate of the mean difference in blood pressure between ICSI and SC children for a certain parameter. Data analysis was performed using SPSS version 19.0 (IBM SPSS Statistics 19). A *P*-value < 0.05 was considered statistically significant.

The present sample size of ~100 females and males in the ICSI and SC groups allowed us to detect a difference in systolic blood pressure of 4 mmHg and a difference in diastolic blood pressure of 2.3 mmHg with a power of 80% at a significance level of 0.05.

Results

Baseline characteristics

Birthweight, birthweight SDS and gestational age were significantly lower in children conceived by ICSI compared with their SC counterparts (3280 ± 496 versus 3436 ± 481 g; −0.3 ± 1.1 versus 0.0 ± 1.3; 38.7 ± 1.3 versus 39.4 ± 1.5 weeks respectively; all $P < 0.01$). The overall number of children born SGA was low, but higher in the ICSI group (8.3%) than in the SC group (4.1%; $P = 0.07$). Mothers of children born after ICSI were older (32.0 ± 4.6 versus 28.5 ± 4.4 years; $P < 0.01$) but not more often highly educated (66 versus 68%; $P = 0.7$). ICSI mothers did not experience more pregnancy-induced hypertension/pre-eclampsia/diabetes in comparison with the mothers who conceived spontaneously (5 versus 4%; $P = 0.4$). The mothers of the ICSI and SC groups had comparable BMI (23.6 ± 3.7 versus 23.3 ± 3.3 kg/m^2; $P = 0.3$) and a comparable risk of having cardiovascular disease (13 versus 14%; $P = 0.9$). More ICSI parents were at risk for cardiometabolic disease (42 versus 28%, $P = 0.03$). ICSI fathers (29%) were more often at risk for cardiometabolic disease in comparison with fathers from SC teenagers (14%; $P < 0.001$). ICSI boys (14.0 ± 0.4 years) and girls (14.0 ± 0.3 years) were on average younger compared with SC boys (14.3 ± 0.3 years) and girls (14.3 ± 0.3 years) at the time of examination ($P < 0.01$). Table I shows an overview of clinical data in our sample of 14-year-old boys and girls.

In girls, a positive correlation was found between body weight and systolic ($r = 0.2$) and diastolic ($r = 0.1$) resting blood pressure (all $P < 0.05$), but no correlations were found between systolic or diastolic blood pressure and height and age (all $P > 0.1$). A statistically significant increase in systolic and diastolic blood pressure was observed across the pubertal stages ($P = 0.02$ and 0.01).

In boys, systolic and diastolic blood pressure correlated positively with body weight ($r = 0.5$ and 0.2), height ($r = 0.4$ and 0.1) and age ($r = 0.3$ and 0.2) (all $P < 0.05$). In boys, a statistically significant increase in systolic blood pressure across the pubertal stages was observed ($P < 0.001$); however, the increase in diastolic blood pressure across the pubertal stages did not reach statistical significance ($P = 0.4$).

In girls and in boys, none of the early life factors (birthweight and gestational age) or parental characteristics (maternal age, maternal educational level, maternal BMI, pregnancy-induced hypertension and parental risk estimates for cardiometabolic disease) were associated with systolic or diastolic blood pressure (data not shown).

Growth during infancy and blood pressure after ICSI conception

ICSI girls and ICSI boys with rapid weight gain either in early infancy (0–3 months) or in late infancy (3–12 months) did not have higher systolic or diastolic blood pressure at age 14 years in comparison with ICSI girls and ICSI boys with slow weight gain in early infancy or late infancy (data not shown).

Resting blood pressure after ICSI conception

In girls, systolic blood pressure was comparable in the ICSI (109 ± 9 mmHg) and the SC groups (111 ± 9 mmHg; $P = 0.2$; Table I). Diastolic blood pressure as well was comparable in the ICSI group (64 ± 6 mmHg) and in the SC group (66 ± 7 mmHg; $P = 0.05$). Correction for height and age in girls did not alter the results for systolic and diastolic blood pressure ($P = 0.1$ and 0.05; Table II).

In boys, systolic blood pressure was slightly lower in the ICSI group (113 ± 10 mmHg) than that the SC group (116 ± 9 mmHg; $P = 0.04$; Table I). Diastolic blood pressure was comparable in ICSI (64 ± 6 mmHg) and in SC boys (65 ± 5 mmHg; $P = 0.1$). After adjustment for height and age, no difference was observed in systolic or diastolic blood pressure between ICSI and SC boys ($P = 0.7$ and 0.6; Table III).

Table I Clinical characteristics of 14-year-old ICSI and SC children stratified by gender

	Boys, ICSI (*n* = 116)	SC (*n* = 115)	*P*	Girls, ICSI (*n* = 101)	SC (*n* = 108)	*P*
Age (years)	14.0 ± 0.4	14.3 ± 0.3	<0.01	14.0 ± 0.5	14.3 ± 0.3	<0.01
Weight SDS	−0.2 ± 1.1	0.0 ± 0.9	0.3	0.0 ± 0.9	−0.2 ± 0.9	0.06
Weight (kg)	53.0 ± 12.1	56.1 ± 11.5	0.04	53.1 ± 8.7	51.6 ± 7.2	0.2
Height SDS	−0.1 ± 1.2	0.3 ± 0.9	0.02	0.2 ± 1.0	0.0 ± 1.0	0.1
Height (cm)	165.8 ± 9.7	170.3 ± 7.8	<0.01	164.0 ± 6.6	163.3 ± 6.8	0.5
BMI SDS	−0.2 ± 1.1	−0.2 ± 1.0	0.9	−0.1 ± 0.9	−0.3 ± 0.8	0.2
BMI (kg/m^2)	19.1 ± 3.1	19.2 ± 3.0	0.8	19.7 ± 2.8	19.3 ± 2.2	0.3
Tanner stage of genital (boys) and breast (girls) development (*n*; %)			0.3			0.04
2	8 (7)	5 (4)		3 (3)	2 (2)	
3	40 (34)	29 (25)		33 (32)	18 (17)	
4	55 (48)	65 (57)		53 (53)	69 (64)	
5	13 (11)	16 (14)		12 (12)	19 (17)	
Blood pressure						
Systolic (mmHg)	113 ± 10	116 ± 9	0.04	109 ± 9	111 ± 9	0.2
Diastolic (mmHg)	64 ± 6	65 ± 5	0.1	64 ± 6	66 ± 7	0.05

Table II Differences in systolic and diastolic blood pressure between ICSI and SC females, after adjustment for current, early life and social factors.

	Systolic blood pressure			Diastolic blood pressure		
	B	95% CI	*P*	*B*	95% CI	*P*
Unadjusted	−1.7	−4.3, 0.8	0.2	−1.7	−3.6, 0.0	0.05
Adjusted for current characteristics						
Age[a]	−1.7	−4.4, 0.9	0.2	1.7	−3.6, 0.1	0.06
Height[a]	−1.9	−4.4, 0.6	0.1	−1.8	−3.6, 0.0	0.05
Weight[a]	−2.1	−4.6, 0.4	0.1	−1.9	−3.7, −0.2	0.03
Pubertal stage (the Tanner score for breast development)[a]	−1.4	−3.9, 1.2	0.3	−1.2	−3.0, 0.6	0.2
Adjusted for age and height	−2.1	−4.8, 0.5	0.1	−1.8	−3.7, 0.0	0.05
Adjusted for all current characteristics	−2.5	−5.3, 0.2	0.07	−1.4	−3.4, 0.5	0.1
Adjusted for early life characteristics[b] (birthweight and gestational age)	−1.8	−4.6, 0.9	0.2	−1.8	−3.7, 0.1	0.06
Adjusted for parental characteristics[b] (maternal age, maternal educational level, maternal BMI, pregnancy−induced hypertension and parental risk for cardiometabolic disease)	−1.9	−5.0, 1.1	0.2	−1.7	−3.9, 0.3	0.1
Adjusted for current, early life and parental characteristics	−1.9	−5.0, 1.3	0.2	−1.1	−3.3, 0.0	0.3

[a]Each row represents a separate regression analysis.
[b]Additional adjustment for age and height.

Table III Differences in systolic and diastolic blood pressure between ICSI and SC males, after adjustment for current, early life and social factors.

	Systolic blood pressure			Diastolic blood pressure		
	B	95% CI	*P*	*B*	95% CI	*P*
Unadjusted	−2.6	−0.1, −5.1	0.04	−1.2	−2.8, 0.3	0.1
Adjusted for current characteristics						
Age[a]	−0.7	−3.2, 1.8	0.6	−0.6	−2.2, 1.0	0.5
Height[a]	−0.6	−2.9, 1.7	0.6	−0.9	−2.5, 0.7	0.2
Weight[a]	−1.4	−3.6, 0.8	0.2	−0.9	−2.5, 0.5	0.2
Pubertal stage (the Tanner score for genital development)[a]	−1.8	−4.2, 0.5	0.2	−1.1	−2.7, 0.4	0.1
Adjusted for age and height	−0.4	−2.0, 2.8	0.7	−0.5	−2.1, 1.1	0.6
Adjusted for all current characteristics	−0.2	−2.5, 2.1	0.9	−0.6	−2.3, 1.0	0.5
Adjusted for early life characteristics[b] (birthweight and gestational age)	−0.1	−2.4, 2.5	0.9	−0.7	−2.4, 1.0	0.4
Adjusted for parental characteristics[b] (maternal age, maternal educational level, maternal BMI, pregnancy-induced hypertension and parental risk for cardiometabolic disease)	−0.5	−3.4, 2.3	0.7	−0.9	−2.9, 1.0	0.3
Adjusted for current, early life and parental characteristics	−1.1	−3.8, 1.6	0.4	−1.2	−3.2, 0.7	0.2

[a]Each row represents a separate regression analysis.
[b]Additional adjustment for age and height.

Resting blood pressure after ICSI conception adjusted for potential confounders

Influences of confounding factors on the difference in blood pressure between ICSI and SC children are shown in Tables II (girls) and III (boys).

After adjustment for all current characteristics (age, height, weight and pubertal stage), systolic and diastolic blood pressure remained comparable between ICSI and SC girls ($P = 0.07$ and 0.1). The unadjusted difference of −1.7 mmHg in systolic blood pressure between ICSI and SC females increased to −2.5 mmHg after adjustment for current characteristics mainly because ICSI females had a higher weight SDS, which is known to be positively correlated with blood pressure. Although the ICSI offspring have a lower birthweight in comparison with the SC offspring and low birthweight is known to be positively correlated with blood pressure, there is only a small and statistically not significant effect of early life factors on blood pressure in our cohort. After adjustment for parental factors (maternal

BMI, maternal educational level, maternal age, pregnancy-induced hypertension and parental risk estimates for cardiometabolic disease) and taking into account age and height of the offspring, the small differences observed for systolic and diastolic blood pressure remained unaltered. After adjustment for current, early life and parental characteristics, ICSI females still showed a comparable systolic and diastolic blood pressure in comparison with SC females.

After adjustment for all current characteristics, both systolic and diastolic blood pressure were comparable between ICSI and SC boys ($P = 0.9$ and 0.5). The attenuation of the unadjusted difference between ICSI and SC boys for systolic (difference −2.6 mmHg) and diastolic (difference (−1.2 mmHg) blood pressure to a difference of −0.2 mmHg for systolic and 0−.6 mmHg for diastolic blood pressure after correction for current factors could be explained by the younger age and the shorter height in ICSI boys. Correction for early life factors, after prior adjustment for height and age, had little effect on the small differences observed for systolic (difference −0.1, $P = 0.9$) and diastolic (difference −0.7, $P = 0.4$) blood pressure in comparison to the results after correction for age and height, showing only a small additional effect of early life characteristics. In parallel, observed differences in resting blood pressure were only slightly affected by adjustment for parental characteristics after initial adjustment for age and height. After adjustment for current, early life and parental characteristics, ICSI boys had a comparable systolic (difference −1.1, 95% CI: −3.8 to 1.6, $P = 0.4$) and diastolic (difference −1.2, 95% CI: −3.2 to 0.7, $P = 0.2$) blood pressure to SC boys.

Continuous blood pressure measurements

In girls and boys, differences in *baseline stress test* values (i.e. the mean of continuous measurements from the baseline period in stress protocol) of heart rate and systolic and diastolic blood pressure between ICSI and SC were not statistically significant after taking into account all current characteristics (age, height, weight and pubertal stage). A statistically significant increase in systolic and diastolic blood pressure and in heart rate was observed during the stress test in all ICSI and SC females and males (data not shown). The systolic and diastolic blood pressure *response to the stress test* were comparable in ICSI and SC adolescents even after taking into account the baseline values (Table IV). Adjustment for all current characteristics did not alter these results.

Continuous blood pressure measurements were only available from 67 ICSI children (40 boys, 27 girls) and from 38 SC children (23 boys, 15 girls). A *post hoc* power analysis showed that in order to detect a difference in stress response of 6 mmHg between the ICSI and the SC groups, with a power of 80% at a significance level of 0.05, 20 subjects in each group are required. This part of the study is therefore underpowered in detecting smaller differences and the negative results should be interpreted with caution.

Table IV Differences in baseline stress test values and stress response between ICSI and SC females and males[a]

	Females		Males	
	ICSI (*n* = 27)	SC (*n* = 15)	ICSI (*n* = 40)	SC (*n* = 23)
Baseline stress test values				
Systolic BP (mmHg)	96 ± 17	117 ± 26	107 ± 24	99 ± 27
Diastolic BP (mmHg)	57 ± 9	70 ± 12	62 ± 11	61 ± 12
Heart rate (bpm)	81 ± 10	85 ± 13	80 ± 12	75 ± 8
Stress response[a]				
Systolic BP (mmHg)	11 ± 20	4 ± 8	6 ± 12	7 ± 13
Diastolic BP (mmHg)	6 ± 7	3 ± 4	4 ± 5	4 ± 7
Heart rate (bpm)	3 ± 5	3 ± 5	3 ± 5	3 ± 6

BP, blood pressure.
[a]The stress response was significant in ICSI and SC females and males, but no significant differences were observed between groups when adjusted for all current characteristics and the baseline value. The results presented in this table should be considered as descriptive given the small sample sizes.

Discussion

This is the first study to describe blood pressure in pubertal ICSI offspring, in contrast to earlier studies among IVF conceived children.

We did not find that, at puberty, ICSI children have higher blood pressure at rest than SC controls. Even after adjustment for potential confounders such as current body size, early life factors and parental background characteristics, ICSI children were not at risk for increased resting blood pressure. In ICSI children, weight gain in early or late infancy was not correlated with blood pressure at the age of 14. Furthermore, we found no evidence for an altered blood pressure response to stress, but this result should be considered as descriptive given the small subsample.

It is well known that juvenile blood pressure is positively related to adult blood pressure (Rosner *et al*., 1977; Brotons *et al*., 1989; Nelson *et al*., 1992). However, it is speculated that this 'tracking' of blood pressure from childhood into adulthood is perturbed during adolescence due to the pubertal growth phase (Voors *et al*., 1979; Labarthe *et al*., 1991; Lever and Harrap, 1992). Indeed, we previously reported higher blood pressure in pre-pubertal 8-year-old ICSI children, but this finding was not confirmed in our study of pubertal 14-year-old ICSI children. Whether different comparison groups at 8 and 14 years explain the different outcomes can unfortunately not be answered. Alternatively, besides a different setting at ages of 8 and 14 years, the use of an automated device rather than a manual sphygmomanometer might explain the different outcomes at the age of 8 and 14 years.

Higher blood pressure in IVF offspring was previously reported by Sakka *et al*. (2010) and Ceelen *et al*. (2008). Sakka *et al*. (2010) described higher blood pressure in a small ($n = 106$) and predominantly pre-pubertal group of IVF children (>65% of the subjects) in comparison with a group of children born after spontaneous conception ($n = 68$). In their study, IVF children were more often multiples, firstborns or born prematurely and hence displayed a lower birthweight which has been abundantly shown to be related to higher blood pressure (Barker, 1992). In addition, a relatively high proportion

(12 versus 6.5–9% estimated from different studies; Diamanti-Kandarakis *et al*., 1999; Asuncion *et al*., 2000) of the IVF mothers were diagnosed with polycystic ovarian syndrome which has been shown to be related to obesity in the offspring (Recabarren *et al*., 2008) and may contribute to an increased blood pressure. In line with this, the proportion of mothers with arterial hypertension was 5-fold higher in the IVF group compared with the control group and it has been shown that offspring form mothers who experience high blood pressure are more likely to have high blood pressure in later life (Taittonen *et al*., 1996). Ceelen *et al*. (2008) described higher systolic and diastolic blood pressure in 8–18-year old IVF children in comparison with an age-and gender matched SC control group born to subfertile parents. Since they did not stratify their results according to pubertal stage, no definite conclusion can be drawn regarding the risk of hypertension at puberty. In addition, the degree and etiology of the subfertility may have varied between the two groups, which may have resulted in residual confounding. Indeed, there has been concern about the possibility that the underlying condition of the subfertility might be associated with adverse health effects (Romundstad *et al*., 2008). It is well known that women undergoing IVF, mostly in case of female factor infertility have a higher risk of pregnancy complications which in turn adversely affect the health of the offspring, than women receiving ICSI because of male factor infertility (Kallen *et al*., 2005a,b). In summary, the fact that our study found no increase in blood pressure after ICSI conception, while other studies describing blood pressure in IVF offspring have found this in the past, may be because of confounding due to female-factor infertility or because of its correlation with metabolic syndrome and hypertension. However, based on the fact that we previously found an increased blood pressure in pre-pubertal children conceived by ICSI, we cannot exclude that the lack of effect found in the current study is due to the timing of the study during puberty.

Given our previous findings and those of others, monitoring blood pressure throughout adolescence is mandatory. In a model of fetal programming, hypertension was maintained in male offspring only after passage through puberty (Alexander, 2003). Furthermore, even slightly altered blood pressure levels may have an impact on public health. A meta-analysis showed that a mean reduction in diastolic blood pressure of 5–6 mmHg correlates with a 35–40% reduction in the incidence of stroke (Hypertension Detection and Follow-up Program Cooperative Group, 1982).

We found no effect on blood pressure stress responsivity in a small study with limited power, thus excluding large effects associated with ICSI conception. Previous investigations among IVF and ICSI conceived children have found other markers of increased cardiovascular risk, including endothelial dysfunction and increased pulse wave velocity (Scherrer *et al*., 2012), increased adiposity (Ceelen *et al*., 2007; Belva *et al*., 2012) and subtle differences in fasting glucose levels (Ceelen *et al*., 2008), although the latter was not confirmed by Sakka *et al*. (2010). Future studies should investigate hypothalamic–pituitary–adrenal axis functioning, which potentially may factor into the common etiology of adiposity, glucose intolerance and hypertension among IVF and ICSI children.

Some methodological weaknesses should be mentioned. Firstly, notwithstanding the findings of a low response rate in the ICSI group and the fact that from more than a quarter of the eligible comparison group (28%; 154 = 143 + 11) no reply or reason for refusal could be obtained, implicating no information on the current health status of these teenagers, non-participating analysis in the ICSI as well in the SC group did not reveal differences between participating and non-participating children regarding clinical characteristics. Secondly, although we included more than 200 14-year-old ICSI children, stratifying the data according to gender, dilutes the sample size and therefore, caution must be taken regarding the interpretation of negative conclusions notably for the findings of blood pressure response, which was only performed in a small subgroup. Although our sample size appears to be appropriate, our results are the first described in ICSI offspring and should be confirmed by others and in larger cohorts. Thirdly, blood pressure readings were performed at different times during the day. Finally, the setting of the clinical examination was slightly different (ICSI children were examined at the hospital in the presence of their parents; SC children were examined outside the hospital and without their parents present), which could lead to an effect on the blood pressure measured.

In conclusion, we did not find a higher resting blood pressure or higher stress response after psychological stress in ICSI-conceived pubertal children. Before firm conclusions can be drawn, our results should be confirmed by others and in larger cohorts. Our study does, however, not detract from the need for long-term follow-up of ART children in order to estimate if they are at risk for cardiovascular morbidity in adult life. Therefore, follow-up of these ICSI-conceived teenagers is scheduled at young adult age.

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Authors' roles

The current study was designed by F.B., M.B., R.C.P and T.J.R. F.B. collected the data. F.B. analyzed the data under the direction of R.C.P 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.

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Conflict of interest

All co-authors except M.B. declared no conflict of interest. M.B.'s institution (UZ Brussel) has received unconditional grants from Organon International, Shering-Plough, IBSA, Merck, Ferring.

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