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# Are ICSI adolescents at risk for increased adiposity?

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**BACKGROUND:** Puberty is a critical period for the development of cardio-metabolic disturbances, including a more central body fat distribution. It is still unclear if IVF and more specifically ICSI, can permanently and detrimentally affect body fat accumulation in the human offspring. Therefore, adiposity and body fat distribution in 14-year-old adolescents born after ICSI were investigated.

**METHODS:** Body composition data, including anthropometry (weight, height and BMI), skinfold thicknesses (peripheral: triceps and biceps skinfolds; central: supra-iliacal and subscapular skinfolds; total: sum of the four skinfolds) and circumferences (waist, mid-upper arm) were compared between 217 ICSI singletons (116 boys, 101 girls) and 223 singletons (115 boys, 108 girls) born after spontaneous conception (SC). ICSI teenagers were part of a previously published ICSI cohort followed since birth; SC controls were recruited from schools in the surroundings.

**RESULTS:** Among all boys, no differences in body composition measurements were found between the ICSI and SC group, taking into account confounding variables. In boys with more advanced pubertal stages, a significantly higher sum of peripheral skinfolds was found in the ICSI group compared with the SC group (difference 3.5 mm, 95% confidence interval 0.3–6.6). In girls, peripheral adiposity assessed by skinfolds and mid-upper arm circumference, and central adiposity assessed by skinfolds and waist circumference as well as total adiposity assessed by BMI, the sum of four skinfold thicknesses and skinfold-derived body fat percentage were significantly higher in the ICSI group compared with the SC group, taking into account confounding variables (all $P < 0.05$). Neither parental nor early life factors could explain the differences.

**CONCLUSIONS:** We found that pubertal ICSI girls were more prone to central, peripheral and total adiposity compared with their SC counterparts. ICSI adolescents with advanced pubertal stages showed more peripheral adiposity. Continued monitoring of body fat patterns in adolescents born after fertility treatment is mandatory in order to assess their risk for developing obesity and its related adverse health effects in adulthood.

**Key words:** ICSI / body composition / puberty / adiposity / adolescents / ART / long-term

## Introduction

It is now well established that obesity and particularly a central fat distribution in adolescence are important risk factors for the development of diabetes and cardiovascular disease in adulthood (Kissebah *et al*., 1982; Daniels *et al*., 1999). Body composition typically changes during puberty with variations in the amount of body fat and its distribution according to gender. Since the prevalence of childhood obesity is increasing worldwide, the determinants of the development of obesity at young ages are a major health concern (Reilly and Dorosty, 1999; Ebbeling *et al*., 2002; Reilly *et al*., 2003; Tudor-Locke *et al*., 2007).

Early life has been identified as a key period in the development of obesity (Dietz, 1994). It has become clear that environmental changes during critical periods in early life are associated with alterations in body composition in adult life, both in humans (Ravelli *et al*., 1976; Rogers, 2003) and in animals (Edwards *et al*., 2005). Moreover, the peri-conceptional period may be of particular relevance for later disease risk. Several studies using mouse models have shown that embryo manipulation and *in vitro* culture conditions are associated with adverse effects including increased body mass and adiposity in the adult progeny (Khosla *et al*., 2001; Summers and Biggers, 2003; Fleming *et al*., 2004; Sjoblom *et al*., 2005; Lonergan *et al*., 2006).

In view of the increasing number of children born after assisted reproduction, it is mandatory to investigate if *in vitro* manipulation of the human embryo may have long- term health consequences (Thompson *et al*., 2002). Children born after *in vitro* culture are more frequently

born small-for-gestational age (SGA) (Jackson et al., 2004; McDonald et al., 2009), which has repeatedly been shown to be predictive for adult metabolic diseases (Barker, 1984; Levy-Marchal and Jaquet, 2004). There is limited knowledge on the body composition of adolescents born after assisted reproductive technology (ART). In early childhood, body composition was found to be comparable between IVF and spontaneously conceived (SC) children (Kai et al., 2006; Miles et al., 2007). Ceelen et al. (2007) described alterations in body fat accumulation in 8–18-year-old IVF children, mainly affecting peripheral body fat mass. However, no data exist on body composition of children conceived by the more invasive ICSI technique.

In this study, we compared adiposity of adolescents born after ICSI with results from their peers born after spontaneous conception. We hypothesized that children born after ICSI might present with a more central body fat distribution at puberty.

# Materials and Methods

## Set-up and study population

The study is a cross-sectional evaluation of the eldest cohort of ICSI children, conceived at the Center for Reproductive Medicine at the UZ Brussel and a group of SC children, recruited from schools in the surroundings.

ICSI and SC children were eligible if they were Caucasian singletons, born after at least 32 weeks of gestation. Children born SGA, defined as having a birthweight less than or equal to −2 Standard Deviation Scores (SDS) were not *per se* excluded.

Two hundred and seventeen ICSI children (116 boys, 101 girls) born after transfer of fresh embryos obtained by ICSI using ejaculated and non-ejaculated sperm, 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. ICSI was predominantly performed because of male factor infertility (86%); other indications were female factor infertility (6%), combined infertility (5%) and unexplained infertility (3%). Of the eligible cohort of 501 ICSI children, 116 could not be reached and 168 refused to participate, yielding a participation rate of 56% (tested/reached). Although the proportion of higher educated mothers was lower (53 versus 66%; $P = 0.01$) in the non-participants, birthweight (3219 ± 574 versus 3280 ± 496 g; $P = 0.2$), gestational age (38.6 ± 1.5 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. Reasons for refusal were either practical reasons (distance to UZ Brussel too far) or difficulties in explaining the aim of the study to their son/daughter since parents had not disclosed the method of conception to their offspring. 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 peers regarding male gender (51 versus 53%, $P = 0.8$), attending general education (70 versus 74%, $P = 0.5$) or current weight (females: 50.9 ± 7.8 versus 53.1 ± 8.7 kg, $P = 0.1$; males: 52.1 ± 9.7 versus 53.0 ± 12.1 kg, $P = 0.6$).

The comparison group consisted of 223 children (115 boys, 108 girls), born after spontaneous conception and without the use of hormonal stimulation. SC children were recruited by letter ($n = 553$) in comparable types of schools as those attended by ICSI children. In Belgium, two types of secondary education exist: general education (middle school, high school and college) and vocational educational (technical education). Three hundred and twenty-seven families agreed to participate; 72 refused participation because of lack of time or interest and 11 parents refused without giving a reason. In case of refusal, minimal information on the current health status was asked and obtained from 60 children (26 males; 34 females) (72%). From 143 children, we received no reply to our invitation. It is not clear if those invitations reached the parents, since letters were distributed anonymously to the pupils at school. Children who met the inclusion criteria and whose parents gave written informed consent ($n = 278$) were planned for examination at a moment to coincide with the scheduled check-up by the school physician. In Belgium, all pupils have a compulsory clinical visit at a school health centre. Those visits are organized by the Pupil Guidance Centre, a national health care centre. Due to practical reasons (informed consent obtained after the scheduled visit or absence of children at the visit because of illness) only 223 children could be examined during the study period. Response rate varied between 50% (agreed/eligible; 278/553) and 68% (agreed/reached; 278/410). Demographic and clinical data of the refusals were comparable with those of the participating children, such as 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, $P = 0.5$) and maternal age (29.3 ± 3.9 versus 28.5 ± 4.4 years, $P = 0.2$). 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 added the available weight data of the ICSI and SC teenagers who refused participation, to 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, either for the females ($P = 0.6$ in comparison with the reported $P = 0.2$ in Table II) or males ($P = 0.04$ in comparison with the reported $P = 0.04$ in Table II).

The set-up of the present study is similar to a previously published follow-up study at the age of 8 years (Belva et al., 2007). Briefly, data from a physical examination were replenished with child's personal and family history data concerning anthropometric, medical and socio-economic characteristics obtained from a written questionnaire completed by the parents. Regarding the parents, maternal and paternal age, current 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 pre-existing diseases unrelated to pregnancy (such as hypertension, hypercholesterolemia, thyroid and diabetes) and pregnancy-induced disorders (hypertension, pre-eclampsia, gestational diabetes and thyroid) were collected retrospectively by means of self-report. 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 children were examined by one single paediatrician (F.B.) who was not blinded to the mode of conception. Weight and height were measured with a standard technique and equipment and recorded to the nearest 0.1 kg and 0.1 cm. BMI was calculated as weight divided by height squared. Pubertal maturity was assessed on the Tanner scale (Tanner and Whitehouse, 1976) for breast development [mammae (M)] in girls and genital development [genital (G)] in boys. Waist circumference, waist-to-hip (W/H) ratio and mid-upper arm (MUAC) circumference were measured using a non-stretchable tape. Waist circumference was measured at the level of the narrowest point between the lower costal border and the iliac crest, with the subjects standing. Hip circumference

was measured at the widest point over the hip and buttocks. The MUAC was measured at a point 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 an index of peripheral adiposity, subscapular and suprailiac skinfold thickness as an index of central adiposity. The sum of the four skinfolds was used as a measure of total adiposity. The percentage of body fat was derived from the skinfolds using Slaughter equation (Slaughter *et al.*, 1988), which is appropriate for both sexes in adolescence (Rodriguez *et al.*, 2005). 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 SDS using national reference curves (Roelants *et al.*, 2009) hence correcting for age and gender. Birthweight was expressed as SDS in order to correct for gestational age and gender.

## Statistical analysis

Continuous data are presented as means with their standard deviation (SD), and compared between the ICSI and SC group with a Student's *t*-test. Categorical data are presented as number of children (events) and percentages; $\chi^2$ or Fisher's exact test were used for comparisons. The association between mode of conception and measures of body composition was assessed by multiple linear regression, adjusted for potential confounders such as current characteristics (age, pubertal stage, school type and frequency of sports), early life factors (birthweight, gestational age and parity) and parental factors (maternal educational level, maternal BMI). Results are expressed as unstandardized regression coefficients with a 95% confidence interval (95% CI), which is an estimate of the difference in size between ICSI and SC children for a certain parameter. Data analysis were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Exploring data from males and females combined, using analysis of variance, with each anthropometric trait as dependent variable, and gender and mode of conception as independent variables, we found a statistically significant effect of gender, we found a statistically significant effect of 'gender', but not of the interaction term 'gender*mode of conception' on all anthropometric measurements (for 'gender' all $P < 0.01$ and for the 'interaction term' $P$ ranging from 0.2 to 0.9). Consequently, according to these gender-specific, but not conception-specific findings and due to the natural course of pubertal development, with an earlier onset of maturation in girls compared with boys, and opposite changes in body composition in girls (increase of body fat) and boys (decrease in body fat) at puberty, we reported our results stratified according to gender.

# Results

## Baseline characteristics

Birthweight, birthweight SDS and gestational age were significantly lower in children conceived by ICSI compared with their SC counterparts (Table I). The overall number of SGA children in this study is low and the proportion of SGA children is higher in the ICSI group. Mothers of children born after ICSI were older but not more often highly educated. They had a comparable BMI and had not experienced more gestational diabetes or pregnancy-induced hypertension compared with mothers of SC children. Table II shows clinical and anthropometric data stratified by gender. ICSI boys and girls were on average younger compared with SC peers at the time of examination. ICSI children were more often found in the early Tanner stages (stages 2 and 3) and SC children in the more advanced Tanner stages (stages 4 and 5), but this difference was only statistically significant in girls.

## Body composition after ICSI conception

Comparable sums of peripheral and central skinfolds and comparable total body fat, calculated from the total sum of skinfolds were found in ICSI versus SC boys as well as in ICSI versus SC girls (Table II). In parallel, body fat distribution assessed by the central to peripheral skinfold ratio was comparable between the ICSI and SC groups (all $P > 0.05$). Waist circumference SDS ($-0.1 \pm 0.8$; $P < 0.01$) and MUAC SDS ($0.2 \pm 0.9$; $P = 0.03$) were higher in ICSI-conceived girls compared with SC girls ($-0.4 \pm 0.8$ and $-0.1 \pm 0.7$, respectively), but not in ICSI-conceived boys ($-0.2 \pm 1.0$; $0.0 \pm 1.1$) compared with SC boys ($-0.3 \pm 0.9$; $-0.1 \pm 1.0$). W/H ratio was significantly lower in ICSI-conceived girls ($0.83 \pm 0.05$) compared with SC-conceived girls ($0.85 \pm 0.04$), but this difference was very small ($P = 0.01$).

**Table I Birth characteristics and socio-demographic data.**

| | ICSI, $n = 217$ | SC, $n = 223$ | *P*-value |
|---|---|---|---|
| Birth characteristics | | | |
| Birthweight (g) | $3280 \pm 496$ | $3436 \pm 481$ | <0.01 |
| Birthweight SDS | $-0.3 \pm 1.1$ | $0.0 \pm 1.3$ | <0.01 |
| Gestational age (weeks) | $38.7 \pm 1.3$ | $39.4 \pm 1.5$ | <0.01 |
| Prematurity (32 w–37 w) (*n*; %) | 11 (5) | 14 (6) | 0.7 |
| Small-for-gestational-age (*n*; %) | 18 (8) | 9 (4) | 0.07 |
| Socio-demographic characteristics | | | |
| Primiparity | 174 (84) | 114 (51) | <0.01 |
| Pregnancy-induced hypertension/diabetes | 12 (5) | 8 (4) | 0.4 |
| Maternal BMI (kg/m$^2$) | $23.6 \pm 3.7$ | $23.3 \pm 3.3$ | 0.3 |
| Maternal age (years) | $32.0 \pm 4.6$ | $28.5 \pm 4.4$ | <0.01 |
| Maternal educational level: higher level | 141 (66) | 152 (68) | 0.7 |

Mean $\pm$ SD or numbers (%).

**Table II Clinical characteristics of 14-year-old ICSI and SC children stratified by gender.**

| | Boys ICSI, *n* = 116 | SC, *n* = 115 | *P*-value | Girls ICSI, *n* = 101 | SC, *n* = 108 | *P*-value |
|---|---|---|---|---|---|---|
| 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 |
| 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 [(*n*) %] | | | 0.3 | | | 0.04 |
| 2 | 8 (7) | 5 (4) | | 3 (3) | 2 (2) | |
| 3 | 40 (34) | 29 (25) | | 33 (33) | 18 (17) | |
| 4 | 55 (48) | 65 (57) | | 53 (53) | 69 (64) | |
| 5 | 13 (11) | 16 (14) | | 12 (12) | 19 (17) | |
| Sport frequency (hours/week) | 4.3 (2.5) | 4.5 (1.9) | 0.5 | 3.0 (2.0) | 3.8 (2.7) | 0.04 |
| School type [(*n*)%] | | | <0.01 | | | 0.7 |
| Secondary school | 80 (69) | 97 (84) | | 81 (80) | 84 (78) | |
| Vocational school | 36 (31) | 18 (16) | | 20 (20) | 24 (22) | |
| Sum of peripheral skinfolds (mm) | 19.7 ± 9.5 | 18.1 ± 9.0 | 0.2 | 26.8 ± 8.5 | 25.2 ± 7.8 | 0.1 |
| Sum of central skinfolds (mm) | 19.2 ± 10.4 | 19.0 ± 9.7 | 0.8 | 27.0 ± 10 | 25.4 ± 8.4 | 0.2 |
| Total sum of skinfolds (mm) | 38.9 ± 19.2 | 37.0 ± 17.7 | 0.4 | 53.8 ± 17.8 | 50.6 ± 15.2 | 0.1 |
| Subscapular/triceps skinfold ratio | 0.83 ± 0.22 | 0.86 ± 0.28 | 0.3 | 0.8 ± 0.3 | 0.8 ± 0.2 | 0.6 |
| Total body fat (%)[a] | 16.6 ± 7.5 | 15.2 ± 6.5 | 0.1 | 22.8 ± 5.2 | 22.0 ± 4.7 | 0.2 |
| Waist circumference SDS | −0.2 ± 1.0 | −0.3 ± 0.9 | 0.5 | −0.1 ± 0.8 | −0.4 ± 0.8 | <0.01 |
| Waist circumference (cm) | 68.3 ± 7.0 | 68.4 ± 6.4 | 0.9 | 66.2 ± 5.4 | 64.7 ± 4.6 | 0.03 |
| Mid-upper arm circumference SDS | 0.0 ± 1.1 | −0.1 ± 1.0 | 0.7 | 0.2 ± 0.9 | −0.1 ± 0.7 | 0.03 |
| Mid-upper arm circumference (cm) | 24.4 ± 5.0 | 24.2 ± 3.4 | 0.8 | 24.8 ± 3.0 | 24.0 ± 2.1 | 0.04 |
| W/H ratio | 0.92 ± 0.05 | 0.91 ± 0.04 | 0.6 | 0.83 ± 0.05 | 0.85 ± 0.04 | 0.01 |

Mean ± SD or numbers (%).
[a]According to Slaughter formula, see Slaughter *et al.* (1988).

## Body composition after ICSI conception adjusted for potential confounders

The association between mode of conception and body fat measures adjusted for current characteristics are shown in Table III for girls and in Table IV for boys.

After adjustment for current characteristics (age, pubertal stage, school type and frequency of sports), ICSI girls had a significantly higher sum of peripheral, central and total sum of skinfolds and a significantly higher mean MUAC and waist circumference in comparison with SC peers (difference between ICSI and SC was 2.8 mm, 3.1 mm, 6.3 mm, 1.5 cm and 2.1 cm, respectively; all $P < 0.05$) (Table III). The BMI and percentage body fat mass were also significantly higher in ICSI girls compared with SC girls (difference between ICSI and SC was 1.1 kg/m$^2$ and 1.8%, respectively; $P < 0.01$). Body fat distribution expressed by W/H ratio was significantly different between ICSI and SC girls, with a lower W/H ratio in ICSI girls compared with SC girls (difference between ICSI and SC was −0.02; $P < 0.05$). Additional correction for early life factors (gestational age, birthweight and parity) and parental factors (maternal BMI, maternal educational level) did not change the observed differences and significance levels of the body fat measures (Table III). An analysis, limited to girls in more advanced pubertal stages (pubertal stages M4 or M5) showed that differences in body fat measurements between ICSI and SC girls were even more pronounced (Table III). On the contrary, early pubertal ICSI and SC girls (pubertal stage M2 and M3) did not exhibit differences in body fat measurements (data not shown).

In boys, no difference in body fat measurements between ICSI and SC was found, after adjusting for current characteristics (all $P > 0.05$) (Table IV). However, in boys with advanced pubertal development (pubertal stages G4 or G5), ICSI boys had a significantly higher sum of peripheral skinfolds (difference between ICSI and SC was 3.5 mm; $P < 0.05$) and tended towards a higher percentage body fat mass (difference between ICSI and SC was 2.1%; $P < 0.05$) and lower central/peripheral skinfold ratio (difference between ICSI and SC was 0.09; $P < 0.05$) compared with SC boys (Table IV).

# Discussion

This study describes body fat mass and distribution assessed by anthropometric measurements in the first and eldest cohort of ICSI children. ICSI girls were found to have increased peripheral, central and total adiposity. ICSI boys with more advanced pubertal development were found to have greater peripheral adiposity and possibly total adiposity and altered body fat distribution.

**Table III Associations between the mode of conception and measures of body fat in girls adjusted for potential confounders.**

| | All pubertal stages | | | | Subanalysis: Advanced pubertal stages (M4 or M5) | |
|---|---|---|---|---|---|---|
| | Adjusted for current characteristics[a] | | Adjustment for current[a] and early life[b] and parental[c] characteristics | | Adjusted for current characteristics[a] | |
| | *B* | 95% CI | *B* | 95% CI | *B* | 95% CI |
| Peripheral adiposity | | | | | | |
| Sum of peripheral skinfolds (mm) | 2.8* | 0.2–5.4 | 2.8* | 0.4–5.3 | 3.7* | 0.5–7.0 |
| Mean mid-upper arm circumference (cm) | 1.5*** | 0.8–2.2 | 1.4*** | 0.7–2.0 | 1.8*** | 1.1–2.6 |
| Central adiposity | | | | | | |
| Sum of central skinfolds (mm) | 3.1* | 0.7–5.4 | 3.4* | 0.7–6.0 | 4.9** | 1.6–8.2 |
| Waist circumference (cm) | 2.1** | 0.8–3.4 | 2.1** | 0.7–3.5 | 2.6*** | 1.1–4.2 |
| Total adiposity | | | | | | |
| Total sum of skinfolds (mm) | 6.3* | 1.3–11.2 | 6.3** | 1.6–11.0 | 8.6** | 2.5–14.7 |
| BMI (kg/m$^2$) | 1.1** | 0.3–1.8 | 1.0** | 0.3–1.6 | 1.6** | 0.7–2.6 |
| Skinfold thickness derived body fat mass (%) | 1.8** | 0.5–3.0 | 1.9* | 0.4–3.3 | 2.6** | 0.8–4.3 |
| Body fat distribution | | | | | | |
| Subscapular/triceps skinfold ratio | 0.03 | −0.04 to 0.1 | 0.02 | −0.1 to 0.1 | 0.07 | −0.05 to 0.2 |
| W/H-ratio | −0.02* | −0.05 to 0.0 | −0.02* | −0.03 to 0.0 | −0.02** | −0.03 to 0.0 |

B, unstandardized regression coefficients.
[a]Current characteristics: age, pubertal stage, school type and frequency of sports.
[b]Early life factors: gestational age, term and parity.
[c]Parental characteristics: maternal BMI, maternal educational level.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

**Table IV Associations between the mode of conception and measures of body fat in boys adjusted for current characteristics.**

| | Adjusted for current characteristics[a] | | | |
|---|---|---|---|---|
| | All pubertal stages | | Subanalysis: advanced pubertal stages (G4 or G5) | |
| | *B* | 95% CI | *B* | 95% CI |
| Peripheral adiposity | | | | |
| Sum of peripheral skinfolds (mm) | 1.2 | −1.4 to 3.8 | 3.5* | 0.3–6.6 |
| Mean mid-upper arm circumference (cm) | 0.6 | −0.7 to 1.8 | 0.4 | −0.7 to 1.5 |
| Central adiposity | | | | |
| Sum of central skinfolds (mm) | 0.5 | −2.4 to 3.3 | 0.9 | −2.3 to 4.3 |
| Waist circumference (cm) | 0.2 | −1.5 to 1.8 | 1.0 | −1.4 to 3.4 |
| Total adiposity | | | | |
| Total sum of skinfolds (mm) | 1.7 | −3.5 to 6.9 | 4.1 | −1.6 to 9.9 |
| BMI (kg/m$^2$) | 0.2 | −0.6 to 1.0 | 0.4 | −0.7 to 1.5 |
| Skinfold thickness derived body fat mass (%) | 1.0 | −1.0 to 3.0 | 2.1* | 0.0–4.3 |
| Body fat distribution | | | | |
| Subscapular/triceps skinfold ratio | −0.02 | −0.1 to 0.1 | −0.09* | −0.1 to 0.0 |
| W/H-ratio | −0.01 | −0.02 to 0.01 | −0.01 | −0.02 to 0.01 |

B, unstandardized regression coefficients.
[a]Current characteristics: age, pubertal stage, school type and frequency of sports.
*$P < 0.05$.

Recently, Ceelen et al. (2007) described an altered body fat distribution in IVF children between 8 and 18 years. A significantly higher peripheral body fat mass, but not central body fat mass and an increase in total body fat mass were found in IVF-conceived children in comparison with age- and gender-matched controls born to subfertile couples. However, the authors did not stratify their results by gender or pubertal stage. Changes in body composition, including body fat percentage and distribution, at adolescence, are sexually dimorphic and more strongly related to pubertal development than to chronological age. More specifically, the percentage body fat mass increases in girls and decreases in boys during puberty, independently of their chronological age (Rogol et al., 2002; Moreno et al., 2003). We were able to show that ICSI girls had a higher percentage body fat mass compared with SC girls and that these differences were even more pronounced in girls with a more advanced pubertal development. This finding is of particular importance since it confirms that a more metabolically unfavourable body composition develops during adolescence. Indeed, it has been repeatedly shown that adolescent body fat mass, and specifically abdominal obesity, is associated with adverse levels of metabolic and cardiovascular risk factors in adulthood (Ebbeling et al., 2002; Despres, 2006;Tirosh et al., 2011). We did not find increased central or total adiposity in ICSI boys in our study. This could indicate that the ICSI procedure amplifies the sexual dimorphism in body fat accumulation and distribution at puberty that is generally observed among 14-year-old teenagers. Although the differences between ICSI boys and SC boys did not reach statistical significance, they point towards the same direction as differences in body composition found in the female population. Since the pubertal onset is known to occur later in boys than in girls, and because total body fat decreases during male puberty with central fat deposition increasing only with age, monitoring males at more advanced ages will add to the understanding of changes in body composition in ICSI males.

The underlying mechanisms linking embryo manipulation and adverse health outcome are not well understood. Nowadays, the role of epigenetics in human ART is increasingly postulated. Manipulation of gametes and *in vitro* culture may lead to altered gene expression of imprinted genes involved in growth and development (Doherty et al., 2000, Young et al., 2001, Lonergan et al., 2006). Suboptimal culture conditions are known to affect gene expression as well as fetal development. In mice, embryo development in culture is associated with increased adult body mass and adiposity in the offspring (Sjoblom et al., 2005). It is acknowledged that for some technologies, such as ICSI, direct epigenetic alteration of gene expression is the most plausible origin of subsequent aberrations in growth and development (Thompson et al., 2002). In a mouse model, the use of ICSI with DNA-fragmented sperm resulted in blastocysts with alterations in the methylation pattern of some imprinted genes and in the development of obesity in the adult offspring (Fernandez-Gonzalez et al, 2008). Alternatively, whether hormonal stimulation alters the intrauterine hormonal environment with an impact on the growing fetus is still an ongoing debate (Rojas-Marcos et al., 2005; Griesinger et al., 2008). Epidemiological studies have shown that environmental changes during sensitive periods can permanently re-program metabolic and endocrine key systems (Barker, 1995; Bateson et al., 2004). Crucial hormones involved in growth and metabolism are the glucocorticoids, insulin, growth hormone and insulin growth factor-1, all of which have been shown to have the potential to mediate effects of environmental factors and to influence programming of target organs (Fowden et al., 2005). In addition, we cannot exclude that rapid post-natal growth or nutritional factors during childhood, which have been associated with excessive weight gain at adolescence, might have differed between the ICSI and the SC group.

Besides the lack of nutritional data some other limitations should be mentioned. Firstly, the golden standard for assessing body fat is the dual-energy X-ray absorptiometry; this was not possible since SC children were examined outside the hospital setting. However, although the accuracy of most equations to estimate the percentage of body fat based on skinfolds in adolescents is generally poor at the individual level, the Slaughter equation has been found to adequately predict body fat percentage in adolescents of both sexes (Rodriguez et al., 2005). Secondly, the response rate in the ICSI group seems low but is comparable to that in other longitudinal studies in IVF and ICSI children (Basatemur et al., 2010). In addition, no differences were found in clinical data in participating and non-participating ICSI children, but the maternal educational level was generally lower in those who did not participate. However, this is not likely to have influenced the results since the same trend was observed in mothers of SC children who refused to participate. Even if selection bias can be ruled out since SC adolescents were recruited in all types of secondary education, participation bias in the comparison group might not be excluded since 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 of the current health status of those teenagers. Nonetheless, non-participating analysis in the comparison group did not reveal differences between participating and non-participating children regarding clinical characteristics. Along with this, overall non-participation of ICSI and SC teenagers did not seem to affect the described differences in weight between the ICSI and SC population, according to a sensitivity analysis. Finally, although we included more than 200 14-year-old ICSI children, stratifying the data according to gender dilutes the sample size. Since the reported anthropometric findings reached statistical significance only in females but not in males, caution must be taken regarding the interpretation of negative conclusions. The present sample size allowed us to detect a difference of approximately 0.35 SDS with a power of 70% at a significance level of 5%.

Despite these limitations, our investigation provides robust cross-sectional data on body composition in ICSI teenagers during pubertal development, especially since all observations were performed by one investigator. Comparing anthropometric measurements from a homogeneous study population in which ICSI was performed predominantly because of male factor infertility with results from peers born to fertile parents, eliminates the degree of subfertility as a possible confounding factor. Although lower birthweight, shorter gestational age and primiparity, all more frequently associated with ART, are themselves known to be related with body composition at later age, the findings of increased adiposity risk described in the female population were robust after correction for these early life characteristics.

In conclusion, pubertal ICSI girls are at risk for an unfavourable body composition, showing an increase in peripheral, central and total adiposity, assessed by skinfolds and circumferences. In ICSI boys only those with advanced pubertal maturation were at risk for increased peripheral adiposity. In view of the widely established obesity–related

co-morbidities at adult age, ongoing follow-up studies of teenagers born after ART are warranted.

## Authors' roles

The current study was designed by F.B., M.B. and J.D.S., F.B. collected the data. F.B. analyzed the data under the direction of M.R. and J.D.S. 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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## References

Barker DPJ. *Mothers, Babies and Disease in Later Life*. London: BMJ Publishing Group, 1984.

Barker DJ. Fetal origins of coronary heart disease. *Br Med J* 1995; **311**:171–174.

Basatemur E, Shevlin M, Sutcliffe A. Growth of children conceived by IVF and ICSI up to 12 years of age. *Reprod Biomed Online* 2010;**20**:144–149.

Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, Gluckman P, Godfrey K, Kirkwood T, Lahr MM *et al.* Developmental plasticity and human health. *Nature* 2004;**430**:419–421.

Belva F, Henriet S, Liebaers I, Van Steirteghem A, Celestin-Westreich S, Bonduelle M. Medical outcome of 8-year-old singleton ICSI children (born ≥32weeks' gestation) and a spontaneously conceived comparison group. *Hum Reprod* 2007;**22**:506–515.

Ceelen M, van Weissenbruch MM, Roos JC, Vermeiden JP, van Leeuwen F, Delemarre-van de Waal H. Body composition in children and adolescents born after in vitro fertilization or spontaneous conception. *J Clin Endocrinol Metab* 2007;**92**:3417–3423.

Daniels SR, Morrison JA, Sprecher DL, Khoury P, Kimball TR. Association of body fat distribution and cardiovascular risk factors in children and adolescents. *Circulation* 1999;**99**:541–545.

Despres JP. Is visceral obesity the cause of the metabolic syndrome? *Ann Med* 2006;**38**:52–63.

Dietz WH. Critical periods in childhood for the development of obesity. *Am J Clin Nutr* 1994;**59**:955–959.

Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod* 2000;**62**: 1526–1535.

Ebbeling CB, Pawlak DB, Ludwig DS. Childhood obesity: public-health crisis, common sense cure. *Lancet* 2002;**360**:473–482.

Edwards JJ, McFarlane JR, Kauter KG, McMillen IC. Impact of periconceptional nutrition on maternal and fetal leptin and fetal adiposity in singleton and twin pregnancies. *Am J Physiol Regul Integr Comp Physiol* 2005;**288**:R39–R45.

Fernandez-Gonzalez R, Moreira P, Perez-Crespo M, Sanchez-Martin M, Ramirez M, Pericuesta E, Bilbao A, Bermejo-Alvarez P, de Dios Hourcade J, Rodriguez de Fonseca F *et al.* Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. *Biol Reprod* 2008;**78**:761–772.

Fleming TP, Kwong WY, Porter R, Ursell E, Fesenko I, Wilkins A, Miller DJ, Watkins AJ, Eckert JJ. The embryo and its future. *Biol Reprod* 2004; **71**:1046–1054.

Fowden AL, Giussani DA, Forhead AJ. Endocrine and metabolic programming during intrauterine development. *Early Hum Dev* 2005; **81**:723–734.

Griesinger G, Kolibianakis EM, Diedrich K, Ludwig M. Ovarian stimulation for IVF has no quantitative association with birthweight: a registry study. *Hum Reprod* 2008;**23**:2549–2554.

Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol* 2004;**103**:551–563.

Kai CM, Main KM, Andersen AN, Loft A, Chellakooty M, Skakkebaek NE, Juul A. Serum insulin-like growth factor-I (IGF-I) and growth in children born after assisted reproduction. *J Clin Endocrinol Metab* 2006; **91**:4352–4360.

Khosla S, Dean W, Brown D, Reik W, Feil R. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biol Reprod* 2001;**64**:918–926.

Kissebah AH, Vydelingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK, Adams PW. Relation of body fat distribution to metabolic complications of obesity. *J Clin Endocrinol Metab* 1982;**54**:254–260.

Levy-Marchal C, Jaquet D. Long-term metabolic consequences of being born small for gestational age. *Pediatr Diabetes* 2004;**5**:147–153.

Lonergan P, Fair T, Corcoran D, Evans AC. Effect of culture environment on gene expression and developmental characteristics in IVF-derived embryos. *Theriogenology* 2006;**65**:137–152.

McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A, Knowledge synthesis group preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. *Eur J Obstet Gynecol Reprod Biol* 2009;**146**:138–148.

Miles HL, Hofman PL, Peek J, Harris M, Wilson D, Robinson EM, Gluckman P, Cutfield W. In vitro fertilization improves childhood growth and metabolism. *J Clin Endocrinol Metab* 2007;**92**:3441–3445.

Moreno LA, Joyanes M, Mesana MI, Gonzalez-Gross M, Gil CM, Sarria A, Gutierrez A, Garaulet M, Perez-Prieto R, Bueno M *et al.* and the AVENA Study Group. Harmonization of anthropometric measurements for a multicenter nutrition survey in Spanisch adolescents. *Nutrition* 2003;**19**:481–486.

Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. *N Engl J Med* 1976;**295**:349–353.

Reilly JJ, Dorosty AR. Epidemic of obesity in UK children. *Lancet* 1999; **354**:1874–1875.

Reilly JJ, Methven E, McDowell ZC, Hacking B, Alexander D, Stewart L, Keinar C. Health consequences of obesity. *Arch Dis Child* 2003; **88**:748–752.

Rodriguez G, Moreno LA, Blay MG, Blay VA, Fleta J, Sarria A, Bueno M, the AVENA-Zaragoza Study group. Body fat measurement in adolescents: comparison of skinfold thickness equations with dual-energy X-ray absorptiometry. *Eur J Clin Nutr* 2005;**59**:1158–1166.

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.

Rogers I. The influence of birth weight and intrauterine environment on adiposity and fat distribution in later life. *Int J Obes Relat Metab Disord* 2003;**27**:755–777.

Rogol AD, Roemmich JN, Clark PA. Growth at puberty. *J Adolesc Health* 2002;**31**:192–200.

Rojas-Marcos PM, David R, Kohn B. Hormonal effects in infants conceived by assisted reproductive technology. *Pediatrics* 2005;**116**: 190–194.

Sjoblom C, Roberts CT, Wikland M, Robertson SA. Granulocyte-macrophage colony-stimulating factor alleviates adverse consequences of embryo culture on fetal growth trajectory and placental morphogenesis. *Endocrinology* 2005;**146**:2142–2153.

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.

Summers MC, Biggers JD. Chemically defined media and the culture of mammalian preimplantation embryos: historical perspective and current issues. *Hum Reprod Update* 2003;**9**:557–582.

Tanner JM, Whitehouse RM. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 1976;**51**:170–179.

Thompson JG, Kind KL, Roberts CT, Robertson SA, Robinson JS. Epigenetic risks related to assisted reproductive technologies: short- and long-term consequences for the health of children conceived through assisted reproduction technology: more reason for caution? *Hum Reprod* 2002;**17**:2783–2786.

Tirosh A, Shai I, Afek A, Dubnov-Raz G, Avalon N, Gordon B, Derazne E, Tzur D, Shamis A, Vinker S *et al.* Adolescent BMI trajectory and risk of diabetes versus coronary disease. *N Eng J Med* 2011;**364**:1315–1325.

Tudor-Locke C, Kronenfeld JJ, Kim SS, Benin M, Kuby M. A geographical comparison of prevalence of overweight school-aged children: the National Survey of Children's Health 2007. *Pediatrics* **120**:e1043–e1050.

Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, Broadbent PJ, Robinson JJ, Wilmut I, Sinclair KD. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. *Nat Genet* 2001;**27**:153–154.