

Growth and other health outcomes of 2-year-old singletons born after IVM versus controlled ovarian stimulation in mothers with polycystic ovary syndrome

Florence Belva^{1,*}, Mathieu Roelants², Sietske Vermaning¹,
Sonja Desmyttere¹, Jean De Schepper³, Maryse Bonduelle¹,
Herman Tournaye⁴, Frederik Hes¹, and Michel De Vos⁴

¹Center for Medical Genetics, Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium ²Environment and Health/Youth Health Care, Department of Public Health and Primary Care, KU Leuven, Leuven, Belgium ³Department of Paediatric Endocrinology, Universitair Ziekenhuis (UZ Brussel), Brussels, Belgium ⁴Center for Reproductive Medicine, Universitair Ziekenhuis Brussel (UZ Brussel), Brussels, Belgium

*Correspondence address. Center for Medical Genetics, Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussels, Belgium. E-mail: florence.belva@uzbrussel.be

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STUDY QUESTION: Does *In vitro* maturation (IVM) of immature oocytes affect health, including growth at 2 years of age, in singletons born to mothers with polycystic ovary syndrome (PCOS)?

SUMMARY ANSWER: This study of 92 singletons born after IVM in mothers with PCOS showed no significant differences in anthropometry and health outcome parameters in comparison with a cohort of 74 peers born after intracytoplasmic sperm injection (ICSI) and conventional controlled ovarian stimulation (COS) in mothers with PCOS.

WHAT IS KNOWN ALREADY: IVM has been used worldwide in women with PCOS. However, the paucity of available data related to children's health following IVM is an important impediment to a more widespread use of the technology. Although previous reports on the neonatal outcome after IVM are generally reassuring, these studies have flaws that hamper the interpretation of outcomes. Moreover, few studies have reported on health outcomes after IVM beyond infancy, and particularly growth data in children born after IVM of immature oocytes from mothers with PCOS are lacking.

STUDY DESIGN, SIZE, DURATION: This single-center cohort study compared anthropometry and health outcomes in 92 singletons born after ICSI of *in vitro* matured oocytes with 74 singletons born after ICSI without IVM (COS). All participants were born to mothers who were diagnosed with PCOS phenotype A, B, C or D and reached the age of 2 years between November 2012 and June 2019. Singletons born after COS were randomly selected for follow-up until young adulthood.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Anthropometric parameters and health status data were prospectively collected at birth, 4 months and 2 years in cohorts of singletons followed since birth. Results were adjusted for neonatal (birthweight z-score, birth order), treatment (day of transfer, number of embryos transferred, mode of transfer) and parental (maternal smoking, age, body mass index (BMI), anti-Müllerian hormone level, PCOS phenotype, gestational diabetes, hypertensive disorder and paternal BMI) characteristics.

MAIN RESULTS AND THE ROLE OF CHANCE: Overall, no differences were found for bodyweight, height and head circumference z-score between IVM and COS children at birth, at 4 months or at 2 years (all $P > 0.05$). In addition, z-scores of waist and mid-upper arm circumference at 2 years were comparable in IVM and COS children. Adjustment for covariates did not change the conclusion. Surgical intervention rate as well as the hospital admission rate were comparable between the IVM and COS group (all $P > 0.05$). The proportion of children born to mothers with metabolically unfavorable PCOS phenotypes (A and C) was comparable in the two groups (52.1% in IVM and 45.9% in COS). Mothers giving birth to a child conceived using IVM were younger than mothers in the COS group but their BMI was comparable.

LIMITATIONS, REASONS FOR CAUTION: Although our study describes the largest cohort to date of singletons born after IVM applied in mothers with well-defined PCOS phenotypes, the current sample size only allowed us to detect moderate differences in anthropometry. Also, follow-up of children born after IVM for indications other than PCOS, for example fertility preservation after cancer diagnosis, is highly recommended.

WIDER IMPLICATIONS OF THE FINDINGS: We did not observe adverse effects of IVM on growth parameters in offspring ~2 years of age compared to COS, but future studies should focus on cardiovascular and metabolic outcomes in these children and adolescents given their mother's PCOS condition.

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Key words: *In vitro* maturation of oocytes / IVM / health / growth / children / childhood / controlled ovarian stimulation / polycystic ovaries / polycystic ovary syndrome

WHAT DOES THIS MEAN FOR PATIENTS?

In vitro maturation (IVM) of oocytes is a laboratory procedure used for the treatment of infertility. In standard treatment, patients need to give themselves hormonal injections for seven days or longer to achieve mature eggs. In our IVM procedure the eggs are removed before they have fully matured. After removing the immature eggs from the woman's ovary, only some of these eggs will achieve maturity in a culture dish in the lab. Women with a condition called polycystic ovary syndrome (PCOS) are suitable for IVM treatment because they have a higher risk of hormone-induced side effects.

When IVM is completed, the eggs develop the possibility of being fertilized faster than they would inside the ovary. Eggs in a woman's ovary take over 1 week to mature.

Because of this, and the artificial conditions of the culture media, which cannot exactly copy those of the ovary, people have had concerns about the health status of children born after IVM. On the other hand, having a mother with PCOS may also have an impact on the health of offspring.

Therefore, our study investigated whether IVM children differ in health and body measurements from children conceived using a standard fertility treatment.

This study showed that body measurements in 2-year-old IVM children did not differ from those in children conceived after standard fertility treatment. Importantly, both groups of children in our study were born to mothers with PCOS. And interestingly, all children in this study, both in the IVM group and the standard fertility treatment group, had a waist circumference that was higher when compared to children in the general population. We think that this might mean that there are potential health issues for children born to mothers with PCOS and that this is not something caused by IVM.

To be more certain about the safety of the IVM procedure, future studies should focus on looking at the health of children who were born after IVM that was carried out for other reasons, not in mothers with PCOS.

Introduction

In oocyte IVM, oocytes are collected from antral follicles and cultured, matured and fertilized *in vitro* (Edwards, 1965). The avoidance of conventional stimulation of follicles to preovulatory sizes, the absence of an ovulatory trigger and thus the elimination of the risk of ovarian hyperstimulation syndrome makes IVM a suitable treatment option for women suffering from polycystic ovary syndrome (PCOS), which affects ~10% of women (Smits et al., 2011).

The widespread implementation of IVM is, however, hindered by lower live birth rates and higher miscarriage rates when compared to conventional ART (Suikkari, 2008). Nevertheless, neonatal outcome data for children born after IVM are available. Unfortunately, studies on the health of IVM children are often flawed which impedes their comparability and makes it difficult to draw sound conclusions. Most studies are based on small sample sizes (Cha et al., 2005; Mikkelsen

2005; Soderstrom-Antilla et al., 2006; Shu-Chi et al., 2006), do not stratify outcomes regarding multiplicity (Cha et al., 2005; Foix-l'Hélias et al., 2014) or do not include a comparison group (Cha et al., 2005; Mikkelsen 2005; Soderstrom-Antilla et al., 2006). More troublesome is the fact that the IVM and comparison groups often have a different infertility background. For example, in the study of Fadini et al. (2012), >17% of the mothers of a singleton born after IVM were diagnosed with PCO (S) while this was only 5% in the intracytoplasmic sperm injection (ICSI) control group. In the study of Foix-l'Hélias et al. (2014), all mothers of children born after IVM had PCOS while the comparison consisted of children born after ICSI, mostly born to couples suffering from male infertility. Other limiting factors are the lack of health data beyond infancy and the lack of a uniform protocol of IVM, resulting in births derived from oocytes that were mature rather than immature at recovery owing to the use of an ovulation trigger. Although Roesner et al. (2017) investigated 2-year-old singletons born after IVM, ICSI and

in vitro fertilization (IVF), only limited biometric data based on very few IVM ($n = 12$), IVF ($n = 11$) and ICSI ($n = 8$) singletons were reported.

The procedure of IVM of immature oocytes gives rise to specific concerns regarding the health of offspring born following this technique. The prolonged culture time *in vitro* might induce epigenetic changes that affect growth, as has been shown both in animals (McEvoy *et al.*, 2000; Kerjean *et al.*, 2003) and in humans (Sunde *et al.*, 2016). Not only the prolonged culture but also the PCOS condition of the mother could have an impact on the linear growth of the offspring (Doherty *et al.*, 2015). Although several studies reported a trend to a higher birthweight among children born after IVM (Buckett *et al.*, 2007; Fadini *et al.*, 2012), it remains largely unknown whether IVM of oocytes affects skeletal growth and body composition beyond the neonatal period, particularly in children born to mothers diagnosed with well-defined PCOS phenotypes.

In this study, we investigated if IVM of oocytes may have compromised the outcome of the offspring. Therefore, we compared anthropometry and health status of a unique cohort of children conceived following IVM with results from peers conceived by ICSI and conventional controlled ovarian stimulation (COS) in mothers with PCOS.

Material and Methods

Set up and study groups

The study design has been previously described by Mostinckx *et al.* (2019). In brief, included pregnancies were established after transfer of embryos generated by either IVM of oocytes or COS in women with PCOS. All singleton pregnancies beyond 20 weeks of gestation were included that resulted from an oocyte retrieval between January 2010 and December 2016. Only one offspring from each patient was included. The study included women between 18 and 36 years of age with PCOS, as defined by a follicle number per ovary ≥ 12 on ultrasound scan (Balen *et al.*, 2003). All patients were diagnosed with a specific PCOS phenotype, i.e. A, B, C or D according to the recent National Institutes of Health 2012 extension of the ESHRE/ASRM 2003 diagnostic criteria. Patients with endometriosis grade III-IV were excluded. Furthermore, cycles with preimplantation genetic testing, with oocyte donation or with use of non-ejaculated sperm were excluded. As previously explained by Mostinckx *et al.* (2019), patients consented to undergo either IVM or COS after discussion with the fertility doctor at the outpatient clinic regarding the risks and benefits of each procedure.

All IVM pregnancies were achieved using oocytes that had been retrieved without hCG priming and matured *in vitro* after 28–40 h of culture. Details of the IVM procedure are described in detail elsewhere (Mostinckx *et al.*, 2019). Briefly, follicular aspirates were collected, supplemented with heparin and filtered. After collection, cumulus–oocyte complexes were washed and transferred to a dish containing IVM medium supplemented with highly purified-hMG, hCG and human serum albumin. Cumulus–oocyte complexes were cultured for 28–40 h at 37°C under 6% CO₂ and 20% O₂, respectively. Insemination of all mature oocytes in this study was carried out using ICSI.

In total, 393 ART pregnancies in patients diagnosed with PCOS were ongoing after 20 weeks. Of these, 164 pregnancies after

IVM resulted in the birth of 160 liveborn singletons and 229 pregnancies after COS resulted in the birth of 225 liveborn singletons (Mostinckx *et al.* 2019).

For this study, singletons living in Belgium (a clinical visit to our center was required) and reaching the age of 2 years between November 2012 and June 2019 were eligible. All children were part of the neonatal cohort described by Mostinckx *et al.* (2019). IVM and COS families were invited for a clinical examination at the Center for Medical Genetics through telephone after they had received a letter explaining the study objectives and design.

Overall, from the group of 160 IVM liveborns, 133 IVM children were contacted and 27 IVM children were not contacted, two children had died in the neonatal period due to prematurity, five families were living abroad, 12 children did not reach the age of 2 years during the follow-up period and from eight children the only available information was their live birth; without any details the latter were considered as lost to follow-up.

From the 225 liveborns after COS, 17 were not eligible for follow-up (one child had died in the neonatal period, 16 families were living abroad). Of the eligible 208 COS children, 74 were participants in the standard longitudinal follow-up program. Owing to the large number of children conceived by ICSI in our center, only one in three liveborns are randomly selected, with replacement, for further follow-up until young adulthood. As for the IVM group, only children living in Belgium are eligible since a clinical visit in the Center for Medical Genetics is a prerequisite.

A comprehensive follow-up program for children born after ART has been set up in our center since the introduction of IVF in clinical practice. Principally, children born after non-conventional techniques, including IVM, are closely monitored, including an additional physical assessment at the age of 1 year. Anthropometric measurements, collected at the age of 1 year in 74/92 IVM children, are not reported due to the lack of comparable data in COS children.

Measurements

Physical examination

Body weight and (standing) height were measured using standard equipment. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m²). Waist circumference and mid-upper arm circumference (MUAC) were measured using a non-stretchable tape. Waist circumference was measured at the narrowest point between the costal margin and the iliac crest, with the subjects standing upright. 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. Weight, height, BMI, waist circumference and MUAC were converted to age and sex adjusted z-scores using Flemish reference curves (Roelants *et al.*, 2009).

Solely for the purpose of completeness, we report major congenital malformations, which are defined as malformations that generally cause functional impairment or require surgical correction (Bonduelle *et al.*, 2002). Here, we report malformations from birth up to the age of 2.5 years. Malformations in neonates born after IVM are reported in detail elsewhere (Mostinckx *et al.*, 2019).

Questionnaires

Parents were asked to complete a questionnaire covering a broad range of parameters related to their personal and their children's health status. This included information on hospital admissions, illnesses and surgical interventions in the child and current parental height and weight.

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 are presented as mean and SD for continuous variables and as the frequency and percentage for categorical variables. Small-for-gestational age (SGA) was defined as birthweight z-score < -2 , whereas large-for-gestational age (LGA) was defined as birthweight z-score > 2 . Weight gain (delta weight z-score) from birth up to childhood was calculated as weight z-score at 2 years minus birthweight z-score. The IVM and COS groups were compared with a Student's *t*-test for continuous variables and a chi-squared test for categorical variables. z-Scores of antropometric data at 2 years were additionally tested with a one-sample Student's *t*-test. Data analysis was performed using SPSS Statistics version 25 (IBM, Chicago, IL, USA).

Since the study was largely underpowered for comparison of expected congenital malformation rates, this outcome is only described and not tested for statistical significance.

Multiple linear regression analysis was used to investigate differences in body measurements between the IVM and COS group, adjusted for covariates. Results are expressed as unstandardized regression coefficients (B) with a 95% confidence interval (CI). Covariates known to affect body size and composition and covariates that differed among groups were included in the final model; these include neonatal characteristics (birth order), treatment characteristics (fresh versus vitrified embryo transfer, day 3/4 versus day 5/6 embryo transfer, single embryo transfer (SET) or double embryo transfer (DET)) and parental characteristics (maternal smoking during pregnancy, maternal age, maternal anti-Müllerian hormone (AMH) level, maternal PCOS phenotype, gestational diabetes, hypertensive disorder, BMI of the mother and BMI of the father). Outcomes at 4 months and 2 years were additionally adjusted for birthweight z-score.

In a second analysis, the data were stratified by day of embryo (day 3/4 versus day 5/6) and mode of transfer (fresh versus vitrified) in order to rule out the impact of treatment modalities other than IVM or COS.

With an estimated sample of 75 children in each group, differences between group means will become statistically significant when they differ by >0.3 times the SD. Because the main outcome parameters are expressed as z-scores, which have a SD of approximately one, differences between groups will become statistically significant when they differ by >0.3 z-scores. Translated to the measurement scale in 2-year-old children, a difference of 0.3 z-scores corresponds to ~ 0.4 kg for weight, 1 cm for length and 0.4 kg/m^2 for the BMI (Roelants et al., 2009).

Results

Participation

In the IVM group, 92 out of 133 (69%) invited children were clinically examined at an average age of 2.3 years. Five families could not be

reached and 36 families refused to participate because of the following: severe mental retardation of the child ($n = 1$), morbidity owing to prematurity ($n = 1$), long travel distance to the center ($n = 4$), no interest in another clinical visit after the previous (reassuring) one at 1 year of age ($n = 14$) or no reason specified ($n = 16$).

From the 136 families approached in the COS group, 41 families refused participation, 11 families could not be reached and for 10 children an appointment was scheduled but canceled a few hours/days before the visit (mainly because of illness of the child). Finally, 74 (54.4%) children in the COS group were examined at our center.

No differences were found between participating IVM children ($n = 92$) and the non-participating IVM children ($n = 41$) when comparing maternal characteristics (age, BMI and AMH levels), neonatal characteristics (birthweight and gestational age) and treatment characteristics (proportion of SET, vitrified embryo transfer and day 3/4 embryo transfer; data not shown). Likewise, when comparing maternal, neonatal and treatment characteristics, no differences were found between participating COS children ($n = 74$) and non-participating COS children (i.e. those that were not selected during randomization or refused or were not reached; data not shown).

Treatment characteristics

In mothers of IVM children, day3/4 embryo transfers (IVM: 72.8% versus COS: 29.7%; $P < 0.001$) and vitrified embryo transfers (IVM: 64.1% versus COS: 35.1%; $P < 0.001$) were more prevalent in the IVM group than in the COS group. More IVM children were born after DET (in contrast to SET) when compared to those in the COS group (39.1% versus 23.0%; $P = 0.03$). Maternal serum AMH level was significantly higher in the IVM mothers ($11.5 \pm 7.2 \text{ ng/mL}$) than in the COS mothers ($7.7 \pm 4.4 \text{ ng/mL}$; $P < 0.001$).

Parental characteristics

No differences were found between the two groups regarding maternal weight gain during pregnancy or maternal and paternal BMI (all $P > 0.05$, Table 1) but IVM mothers were on average 1.7 years younger than COS mothers ($P = 0.002$) and less likely to smoke ($P = 0.04$). Comparable proportions of the mothers were diagnosed with a metabolically unfavorable PCOS phenotype (type A and C) in the two groups (in IVM: 52.1% and in COS: 45.9%) ($P = 0.42$; Table 1). None of the mothers had phenotype B.

Characteristics of the study population

Mean gestational age was comparable between the IVM (38.8 ± 1.7 weeks) and the COS children (38.9 ± 2.0 weeks) ($P = 0.8$) and the prematurity rate (< 37 weeks) was also comparable (IVM: 15.4% versus COS: 8.1%) ($P = 0.2$). No children were born SGA and a comparable proportion of IVM and COS children was born LGA (7.7% versus 2.7% respectively; $P = 0.2$).

Major malformations were detected in four (4.3%) children born after IVM. Two major malformations (unilateral symbrachydactyly and hypospadias) were detected in the neonatal period and two were detected in the follow-up period (bilateral cryptorchidism and arachnoid cyst).

Table I Parental and treatment characteristics of the study population.

	IVM <i>n</i> = 92	COS <i>n</i> = 74	P**
Parental characteristics*			
Maternal smoking in pregnancy (<i>n</i>)	0	4 (5.4%)	0.04
Weight gain during pregnancy (kg)	11.1 (7.4)	13.3 (7.5)	0.06
Primiparity (<i>n</i>)	77 (83.7%)	61 (82.4%)	0.83
Hypertensive disorder ^o	21 (22.8%)	9 (12.2%)	0.10
Gestational diabetes	7 (7.6%)	9 (12.2%)	0.43
Maternal age at oocyte retrieval (years)	28.5 (3.5)	30.2 (3.3)	0.002
Maternal AMH level (ng/mL)	11.5 (7.2)	7.7 (4.4)	0.000
Current maternal BMI (kg/m ²)	25.4 (5.5)	25.2 (4.9)	0.80
Maternal BMI >25 kg/m ² (<i>n</i>)	43/90 (47.8%)	30/73 (41.1%)	0.43
Current paternal BMI (kg/m ²)	26.0 (5.3)	25.2 (3.8)	0.32
Paternal BMI >25 kg/m ² (<i>n</i>)	31/74 (41.9%)	29/63 (46.0%)	0.73
PCOS phenotype (<i>n</i>)			0.000
Type A	44 (47.8%)	14 (18.9%)	
Type B	0	0	
Type C	4 (4.3%)	20 (27.0%)	
Type D	44 (47.8%)	40 (54.1%)	
Metabolically unfavorable phenotypes (A and C)	52.1%	45.9%	0.42
Treatment characteristics*			
SET (<i>n</i>)	56 (60.9%)	57 (77.0%)	0.03
Vitrified embryo transfer (<i>n</i>)	59 (64.1%)	26 (35.1%)	0.000
Day 3/4 embryo transfer (<i>n</i>)	67 (72.8%)	22 (29.7%)	0.000

AMH: anti-Müllerian hormone; COS, controlled ovarian stimulation; HELLP, hemolysis elevated liver enzymes and low platelets; PCOS, polycystic ovary syndrome; SD, standard deviation; SET, single embryo transfer.

^oHypertensive disorders including pregnancy-induced hypertension, (pre-)eclampsia and HELLP syndrome.

*Continuous data: mean (SD) and categorical data: number (%).

**Chi-square test or Student's *t*-test, as appropriate.

Major malformations were detected in two (2.7%) children born after COS. One infant was diagnosed with a coarctatio aortae and a single kidney and one child was diagnosed with Silver-Russell syndrome during the follow-up period.

Anthropometric outcome

Weight, height and head circumference z-score were comparable between the two groups at birth, at 4 months and at 2 years (Table II). In addition, at 2 years of age, waist circumference and mid-upper arm circumference z-score were not significantly different between the IVM and COS group. Adjustment for neonatal, treatment and parental characteristics did not change this conclusion.

Also, weight gain from birth ~2 years was comparable between the IVM and the COS group, even after adjustment for neonatal, treatment and parental characteristics (B: -0.5, 95% CI: -0.9; 0.1).

At 2 years of age, the z-scores for mean weight, height and BMI were not statistically different from zero in the IVM and COS groups (all $P > 0.05$). However, the mean z-score was significantly larger than zero for waist circumference in the IVM and COS group (both $P < 0.001$), and for the MUAC in the IVM group ($P = 0.01$) but not in the COS group ($P = 0.06$).

Stratification of results according to type of embryo transfer (cleavage stage versus blastocyst), mode of transfer (fresh versus vitrified) and number of embryos

z-Scores of weight, height, BMI, waist circumference and MUAC at 2 years of age were comparable between IVM and COS children, either in the subgroup of children born after cleavage-stage embryo transfer (IVM: $n = 67$, COS: $n = 22$); after fresh embryo transfer (IVM: $n = 33$, COS: $n = 48$); and after SET (IVM: $n = 36$, COS: $n = 17$) or the subgroup of children born after blastocyst transfer (all $P > 0.05$); after vitrified embryo transfer (all $P > 0.05$); and after DET (all $P > 0.05$).

Major health events outcome

Surgery had been performed in 11 (12.0%) children born after IVM and in five (6.8%; $P = 0.3$) children born after COS. Indications for surgical intervention were mostly minor, such as adenotonsillectomy and/or placement of grommets in 5/11 IVM children. Other interventions were as follows: hypospadias correction ($n = 1$), orchidopexy ($n = 1$), circumcision ($n = 3$) and surgery for symbrachydactyly ($n = 1$) in the IVM group; and circumcision ($n = 2$), placement of grommets ($n = 1$),

Table II Anthropometry of IVM and COS children at birth, at 4 months and at 2 years.

	IVM n = 92	COS n = 74	Unadjusted mean difference, 95% CI	P	Adjusted* mean difference, 95% CI	P
At birth						
Child gender male (n)	45 (48.9%)	37 (50%)				
Birthweight (g)	3391 (504)	3306 (579)	85 (−80;250)	0.31	38 (−176;252)	0.72
Birthweight z-score	0.5 (0.9)	0.4 (0.9)	−0.2 (−0.1;0.5)	0.21	0.2 (−0.2;0.6)	0.29
Birth length (cm)	50.4 (2.2)	50.0 (2.8)	0.4 (−0.3;1.2)	0.27	−0.2 (−1.2;0.8)	0.70
Birth length z-score	0.9 (1.0)	0.7 (1.1)	0.2 (−0.1;0.5)	0.18	0.0 (−0.4;0.4)	0.99
Head circumference (cm)	34.1 (1.6)	34.2 (1.8)	−0.1 (−0.6;0.5)	0.80	−0.3 (−1.1;0.4)	0.36
Head circumference z-score	0.4 (1.2)	0.5 (1.0)	−0.1 (−0.5;0.3)	0.60	−0.1 (−0.6;0.4)	0.61
At 4 months						
Age (years)	0.4 (0.2)	0.4 (0.1)				
Weight z-score	0.2 (1.0)	0.1 (1.1)	0.1 (−0.2;0.4)	0.52	0.0 (−0.4;0.4)	0.90
Height z-score	0.1 (1.0)	0.1 (1.1)	0.0 (−0.3;0.4)	0.74	−0.2 (−0.6;0.1)	0.25
Head circumference z-score	0.1 (1.0)	−0.1 (1.0)	0.2 (−0.1;0.6)	0.14	0.2 (−0.2;0.6)	0.41
At 2 years ^o						
Age (years)	2.3 (0.2)	2.3 (0.3)				
Weight z-score	0.1 (1.1)	0.0 (1.2)	0.1 (−0.2;0.5)	0.55	−0.3 (−0.7;0.2)	0.22
Height z-score	0.1 (1.1)	0.0 (1.1)	0.1 (−0.3; 0.4)	0.71	−0.4 (−0.9;0.0)	0.05
BMI z-score	0.0 (1.0)	−0.1 (1.2)	0.1 (−0.2;0.4)	0.55	0.0 (−0.4;0.5)	0.87
Head circumference z-score	0.2 (0.8)	−0.1 (0.9)	0.3 (0.0;0.5)	0.07	0.1 (−0.3;0.5)	0.49
Waist circumference z-score	0.6 (1.2)	0.7 (0.9)	−0.1 (−0.5;0.2)	0.42	−0.4 (−0.9;0.0)	0.07
Mid-upper arm circumference z-score	0.2 (0.9)	0.2 (0.9)	0.0 (−0.3;0.3)	0.87	−0.1 (−0.5;0.3)	0.64

AMH, anti-Müllerian hormone; CI, confidence interval; COS, controlled ovarian stimulation; IVM, *In vitro* maturation; PCOS, polycystic ovary syndrome.

^oAdditional adjustment for birthweight z-score.

*Adjusted for neonatal (birth order), treatment (number of embryos transferred, mode of transfer and day of embryo transfer), parental characteristics (maternal smoking during pregnancy, maternal age, maternal AMH level, maternal PCOS phenotype, gestational diabetes, hypertensive disorder, BMI of the mother and BMI of the father).

cardiac surgery ($n = 1$) and a dermal cyst excision ($n = 1$) in the COS group.

None of the IVM children was admitted to a Neonatal Intensive Care Unit (NICU) for >4 weeks compared with one child in the COS group ($P = 0.26$).

Up to the age of 2.5 years, 23 children born after IVM (25%) had been hospitalized at least once (>1 day, NICU excluded), mainly for infectious diseases ($n = 19$), but also for allergy ($n = 3$) and monitoring after an apparent life-threatening event ($n = 1$). In total, 14 COS children (18.9%) were hospitalized at least once (>1 day, NICU excluded), also mainly for infectious diseases ($n = 11$) as well as for apnea ($n = 2$) and observation after an injury ($n = 1$). The rate of hospital admission was comparable between the two groups ($P = 0.45$).

Discussion

This study describes the general health status, including growth from birth ~2 years of age, in the largest cohort to date of singletons born after non-hCG triggered IVM of immature oocytes from mothers with PCOS. Anthropometry at birth, at 4 months and at 2 years, was found to be comparable in IVM and COS children even after adjustment for neonatal, treatment and parental characteristics. Furthermore, and reassuringly, the rates of surgical interventions and hospital

admissions did not differ between IVM children and peers born after COS.

z-Scores for anthropometric measurements at 2 years of age did not differ between the IVM and COS group, but waist circumference z-scores were significantly higher than zero (the population mean) in both groups. Whether this finding confirms the abundantly reported overall adverse effect of maternal PCOS on the cardiometabolic health of offspring remains unclear (Sir-Petermann *et al.*, 2007, 2009; Kent *et al.*, 2008; Recabarren *et al.*, 2008; Battaglia *et al.*, 2009; Maliqueo *et al.*, 2009; Hanem *et al.*, 2018; Vanky *et al.*, 2019), but it certainly calls for follow-up, especially because waist circumference is a widely accepted measure of central adiposity and hence a suitable screening tool for cardiometabolic risk factors in children (Sardinha *et al.*, 2016).

Our auxiological findings at the age of 2 years are in line with those from Roesner *et al.* (2017) who described development in 2-year-old IVM singletons, albeit in a very small group of only 12 IVM singletons and eight peers born after ICSI. However, Roesner *et al.* (2017) did not adjust for age or gender and did not provide (crucial) information regarding the maternal condition or the precise indication for IVM. This is a frequent drawback in studies on IVM outcome, together with the inclusion of comparison groups with largely different backgrounds (Fadini *et al.*, 2012; Foix-L'Hélias *et al.*, 2014). Since IVM has been performed for distinct conditions, it is of utmost importance to include

study and control groups that are comparable, apart from the IVM procedure itself. Indeed, describing the impact of infertility treatments on offspring health is complex since many factors, including infertility patients' genetic makeup, the laboratory environment or procedures and maternal and paternal characteristics, can affect the outcome (von Versen-Höynck *et al.*, 2019; Castillo *et al.*, 2019; Khandwala *et al.*, 2018).

In most other studies an 'hCG-primed' approach has been adopted, the oocyte of origin (*in vitro* versus *in vivo* matured, respectively) cannot unequivocally be assigned, and an unknown proportion of 'IVM' children have not been conceived using *in vitro* matured oocytes. We, on the contrary, did not use any hCG-trigger and hence all children in our study are born after IVM of immature oocytes. The current and previous findings obtained at the neonatal age provide reassurance regarding the practice of IVM of immature oocytes and offspring health ~2 years of age.

Even though the stratification according to PCOS phenotype in the mothers is unique, which enabled us to compare study subjects and controls with a highly similar background, some limitations should be noted. The apparent differences in treatment characteristics (mainly the mode and type of embryo transfer) between the two groups are due to the dynamics in embryo transfer policy over the years in our center and could have affected the anthropometric outcomes. However, while vitrified embryo transfer (versus fresh embryo transfer) as well as blastocyst (versus cleavage stage embryo) transfer are known to affect weight and LGA rate (Beyer and Griesinger, 2016; Makinen *et al.*, 2013; Hann *et al.*, 2018), our stratified analysis, albeit in small subgroups, did not point to any impact of these characteristics on our results. A matched cohort study design that accounts for potential treatment and parental confounders would have strengthened our conclusions but was not feasible because matching for several parameters, including embryo freezing or not, number of embryos transferred, maternal BMI or PCOS phenotype, would require a number of candidate control children that largely exceeds the 208 that were available. Also, in case of drop-out of control children during the project, we would also have been forced to remove the study (case) child as well, or end up with an unbalanced design. Nevertheless, we believe that selection bias is unlikely since there were no differences in maternal, neonatal and treatment characteristics between the participating and non-participating IVM and COS groups. On the other hand, it should be acknowledged that the composition of the study group is a direct consequence of the fact that women were offered the choice to undergo either IVM or COS, although IVM as a treatment option was probably more frequently discussed with patients presenting with more severe symptoms. Furthermore, given the convincing evidence of the short- and long-term impact of maternal PCOS condition on the health of offspring (see review by Vanky *et al.*, 2019), research should be expanded to include cardiovascular and metabolic outcomes in children and teenagers. In view of this, the findings of a recently published study regarding cardiovascular and metabolic health in spontaneously conceived children from women with PCOS are appealing; although no differences were found in biometry, subtle cardiovascular and metabolic abnormalities were detected at an age as early as 2.5 years and also at 6–8 years in children whose mother was diagnosed with PCOS in comparison with a population-based reference cohort (de Wilde *et al.*, 2018). Finally, by including a second control group of children born after spontaneous conception to mothers with PCOS, we could have investigated better the effect of the technique of IVM

per se and this would have added to the generalisability of our findings. In addition, future studies should also focus on the follow-up of children born after IVM performed in patients in need of fertility preservation after a cancer diagnosis, which is another emerging indication for IVM.

In conclusion, in our study of 166 children born to mothers with PCOS we did not find an adverse effect of IVM on body size and general health outcome parameters ~2 years of age when compared to COS. However, given the known cardiovascular and metabolic implications for children born to mothers with PCOS, continued follow-up of these children born after IVM is warranted.

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Authors' roles

The study was designed by F.B., M.B., J.D.S. and H.T. Data of the mothers were provided by M.D.V. Data of the children were collected by S.V., S.D. and were analyzed 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 and they approved the definitive version of the manuscript.

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Conflict of interest

All co-authors except M.B., M.D.V. and H.T. declared no conflict of interest. M.B. has received consultancy fees from MSD, Serono Symposia and Merck. M.D.V. has received fees for lectures from MSD, Ferring, Gedeon Richter and Cook Medical. H.T. has received consultancy fees from Gedeon Richter, Merck, Ferring, Abbott and ObsEva. The Universitair Ziekenhuis Brussel (UZ Brussel) and the Center for Medical Genetics have received several educational grants from IBSA, Ferring, MSD and Merck for establishing the database for follow-up research and organizing the data collection.

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