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ARTICLE

Increased risk of metabolic dysfunction in children conceived by assisted reproductive technology

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Abstract

Aims/hypothesis Assisted reproductive technology (ART) is the most widely used treatment for infertility and has resulted in millions of births worldwide. The safety of the offspring has been of the utmost concern. Previous studies suggested an increase in metabolic disorders in offspring later in life. The aim of the present study was to investigate metabolic changes at age 6–10 years in offspring conceived as a result of in vitro fertilisation/intracytoplasmic sperm injection (IVF/ICSI).

Methods A total of 380 children born from IVF/ICSI and a matched control group of 380 naturally conceived children, all aged 6–10 years, were recruited. Anthropometric measures, ultrasound and serum tests were performed for body mass, glucose metabolism and lipid profiles, and examination of vasculature structure.

Results The children conceived by ART showed significantly higher fasting blood glucose and serum insulin levels and HOMA-IR (adjusted β [95% CI]: fasting blood glucose 0.49 [0.42, 0.55]; $\log_e$-transformed insulin 0.28 [0.20, 0.35]; $\log_e$-transformed HOMA-IR 0.38 [0.30, 0.46]), as well as a lower HOMA-B and serum apolipoprotein A (ApoA) levels (adjusted β [95% CI]: $\log_e$-transformed HOMA-B −0.19 [−0.27, −0.11]; ApoA −0.17 [−0.21, −0.13]), when compared with the control group. Furthermore, the ultrasound scan indicated elevated carotid intima–media thickness in children conceived by ART (β 0.13 [95% CI 0.12, 0.13]).

Conclusions/interpretation Children conceived by IVF/ICSI have a less favourable glucose and cardiovascular metabolic profile in childhood when compared with naturally conceived children. The underlying mechanisms and potential long-term consequences need to be elucidated in future studies.

Keywords Assisted reproductive technology · Children · Metabolic dysfunction

Linlin Cui and Wei Zhou are joint first authors.

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Research in context

What is already known about this subject?

- Assisted reproductive technology (ART) has resulted in millions of births worldwide
- Previous studies found increased metabolic aberrations in offspring conceived by ART

What is the key question?

- Are there any metabolic changes at age 6–10 years in offspring conceived by in vitro fertilisation/intracytoplasmic sperm injection?

What are the new findings?

- Children conceived by ART showed significantly higher fasting blood glucose and serum insulin levels, higher HOMA-IR, lower HOMA-B and lower serum apolipoprotein A levels in comparison with naturally conceived children
- Ultrasound scans indicated elevated carotid intima–media thickness in children conceived by ART

How might this impact on clinical practice in the foreseeable future?

- Increased metabolic and cardiovascular risk in children conceived by ART is an important finding indicating that continuous monitoring and early intervention for this population should be given full consideration

Abbreviations

ApoA	Apolipoprotein A
CIMT	Carotid intima–media thickness
FBG	Fasting blood glucose
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilisation
PCOS	Polycystic ovary syndrome

Introduction

In recent decades, in vitro fertilisation (IVF) treatment has advanced markedly and approximately 8 million births have occurred as a result [1]. Currently, 1–3% of births in western countries and 1.7–5% of births in countries such as China and Japan are the product of IVF [2].

Different from natural conception, during the IVF procedure gametes and embryos are exposed to a non-physiological environment, including in vitro manipulation, culture media and supraphysiological levels of steroid hormones (e.g. oestradiol). Adaptation to these environmental stimuli during the periconceptional period may lead to a permanent reprogramming of growth and metabolic systems in the offspring and result in long-lasting consequences in their later life.

Some evidence has been obtained from animal studies. Previous studies found that embryo culture components could impact the body mass and adiposity of adult progeny in mice [3]. Calves conceived by IVF displayed increased food intake and growth rate compared with those conceived by artificial insemination even when they shared comparable birthweight [4]. Intriguingly, a human study demonstrated a similar pattern in children conceived by IVF. These children showed a significant catch-up growth period during late infancy and early childhood, associated with long-term body adiposity manifested as increased skinfold thickness [5]. The same study group also reported higher fasting glucose levels but similar BMI and insulin sensitivity in offspring conceived by IVF when compared with offspring conceived naturally [6, 7]. A recent meta-analysis suggested that fasting glucose and insulin levels were higher in offspring born after IVF than after natural conception [8, 9]. These results highlight the importance of glucose and cardiovascular metabolic monitoring in offspring conceived by IVF.

Therefore, we examined diabetes and insulin sensitivity determinants, lipid profiles and carotid intima–media thickness (CIMT) in 6- to 10-year-old children born as a result of IVF and natural conception. We intend to determine whether offspring conceived by IVF had a higher risk of metabolic disturbance in childhood.

Methods

Study population All children conceived after ART (IVF or intracytoplasmic sperm injection [ICSI]) at the Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University are prospectively followed from birth onwards. From November 2017 to February 2019, 885 parents of children aged 6–10 years were invited to attend our reproductive centre for a medical examination; 382

families agreed to participate in our study. Two children were excluded because they offered non-fasting blood samples. The naturally conceived children were recruited from a primary school in Shandong, China. They were matched 1:1 by maternal age at birth (±2 years), sex and age of children to the children conceived by ART. The final study population consisted of 380 children conceived by ART and 380 children conceived naturally.

At enrolment, sociodemographic information and information on lifestyle, dietary intake and medical history of parents were collected using a standardised questionnaire at a face-to-face interview. All children included in our study were singleton births and free from cardiovascular or endocrine diseases. Signed informed consent was obtained from all parents and agreement was also obtained from all children. The study was approved by the ethics committee at the Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University.

Growth and CIMT Height (to the nearest 0.1 cm) and weight (to the nearest 0.1 kg) were measured twice using a stadiometer and scale with calibrated electronic scale. All children and parents were dressed in light clothes. Parent and child height and weight were measured at the same time. BMI was calculated using these two measurements. Waist circumference was measured twice using a tape measure. The mean of these measurements was used. A clinically validated electronic BP monitor (Omron HEM-7012, Omron Healthcare, Japan) was used to measure BP three times on the right arm while the child was seated. The mean value of the last two readings was used. The anterior and posterior CMIT of bilateral carotid arteries was measured using a portable ultrasound machine with an S4-2 linear transducer of 2–4 MHz (CX30; Philips, USA). The sonographer was blinded to participant information. The mean of measurements was used for further analysis.

Biochemical analysis Fasting blood samples were collected in the morning and stored at −80°C until analysis. Fasting blood glucose (FBG) was measured using the hexokinase method (Cobas c702 instrument; Roche Diagnostics, Mannheim, Germany). Fasting insulin was determined in serum by an electrochemiluminescence immunoassay (Cobas e601 instrument; Roche Diagnostics). Total cholesterol, triacylglycerol, HDL-cholesterol and LDL-cholesterol were determined in serum using homogeneous assay (Cobas c702 instrument; Roche Diagnostics). Apolipoproteins were determined in serum using immunoturbidimetry (Cobas c702 instrument; Roche Diagnostics).

Statistical analysis Characteristics of the ART and natural-conception groups were compared by χ^2 test for categorical variables and Wilcoxon rank-sum test for non-normally distributed continuous variables. Multiple linear regression models were used to investigate the associations of ART with CIMT and metabolic measurements. Potential confounders were examined separately by regression analysis, including parental BMI, parental education level, birthweight, gestational age, duration of breastfeeding, dietary factors, physical activity time and sleep time. Factors that changed the crude difference by more than 10% were considered as confounders and included in the final regression model. Given that the distribution of insulin, HOMA-IR and HOMA-B were skewed, $\log_e$ transformation was conducted prior to the application of regression models. All analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC, USA).

Results

Table 1 presents the parental and perinatal characteristics, and dietary and lifestyle factors for the two groups of children (naturally conceived and conceived by ART). Maternal age and BMI, and paternal BMI were significantly higher in the ART group than in the natural conception group. Birthweight was higher and duration of breastfeeding was longer in the ART group. The median age of both groups of children was 7 years and 52.4% of the children were boys. Systolic and diastolic BP was higher in the children conceived by ART. The children conceived by ART had a higher intake of western-style food, marine products and nuts and a lower intake of dairy and vegetables. The sleep duration of children in the ART group was shorter. Gestational age, fruit intake and duration of physical activity were similar in the two groups. A comparison between characteristics of the included ART population (n=380) vs the ART population lost to follow-up (n=503) is shown in ESM Table 1 (maternal age was lower in the study group; there was no significant difference in maternal or paternal BMI before pregnancy, parental education level, gestational age and birthweight between the two groups).

The associations between ART and metabolic variables are shown in Table 2 and Fig. 1. The linear regression models showed that the children conceived as a result of ART (vs naturally conceived children) displayed higher FBG and fasting serum insulin levels, higher HOMA-IR, lower HOMA-B and lower serum levels of total cholesterol and apolipoprotein A (ApoA) (unadjusted β [95% CI]: FBG 0.49 [0.42, 0.55]; adjusted β [95% CI]: $\log_e$ insulin 0.28 [0.20, 0.35]; $\log_e$ HOMA-IR 0.38 [0.30, 0.46]; $\log_e$ HOMA-B −0.19 [−0.27, −0.11]; total cholesterol −0.42 [−0.55, −0.29]; ApoA −0.17 [−0.21, −0.13]). Compared with naturally conceived children, those conceived by ART displayed significantly greater CIMT (β 0.13 [95% CI 0.12, 0.13]). We further stratified the population into two groups according to maternal BMI (≥23 kg/m^2 and <23 kg/m^2). In both BMI groups the children conceived by ART

Table 1 Characteristics of the study population

Characteristic	NC	ART	*p* value
No. of participants	380	380	
Parental characteristics			
Maternal age at delivery, years	30 (27, 33)	31 (28, 33)	0.02
Maternal BMI, kg/m^2	22.3 (20.8, 24.2)	24.5 (22.4, 26.7)	<0.001
Paternal BMI, kg/m^2	25.1 (23.1, 29.3)	26.0 (24.2, 28.1)	<0.001
Maternal or paternal education level (college or higher), n (%)	108 (28.4)	89 (23.4)	0.12
Perinatal characteristics			
Birthweight, kg	3.40 (3.10, 3.65)	3.45 (3.20, 3.80)	0.02
Gestational age at birth, *n* (%)			0.15
<37 weeks (preterm)	20 (5.3)	20 (5.3)	
37–42 weeks (full-term)	349 (92.1)	357 (94.0)	
>42 weeks (post-term)	10 (2.6)	3 (0.8)	
Duration of breastfeeding, *n* (%)			<0.001
Never	6 (1.6)	13 (3.4)	
0–3 months	6 (1.6)	21 (5.5)	
3–5 months	24 (6.3)	17 (4.5)	
6–12 months	147 (38.7)	56 (14.7)	
>12 months	197 (51.8)	273 (71.8)	
Children's characteristics and lifestyle factors			
Age, years	7 (6, 8)	7 (6, 8)	-
Male sex, *n* (%)	199 (52.4)	199 (52.4)	-
Systolic BP, mmHg	103 (98, 109)	105 (101, 109)	<0.001
Diastolic BP, mmHg	62 (58, 66)	67 (64, 70)	<0.001
Intake of food and drink, *n* (%)			
Breakfast daily	353 (92.9)	358 (94.2)	0.46
Dairy food daily	259 (68.3)	193 (50.8)	<0.001
Fruit twice or more daily	117 (30.8)	124 (32.6)	0.59
Vegetables twice or more daily	294 (77.4)	240 (63.2)	<0.001
Soft drinks once or more monthly	120 (31.6)	132 (34.7)	0.36
Western-style food twice or more weekly	8 (2.1)	25 (6.6)	0.003
Marine products once or more weekly	125 (32.9)	214 (56.3)	<0.001
Nuts once or more weekly	142 (37.4)	194 (51.1)	<0.001
Physical activity ≥1 h/day, *n* (%)	152 (40.4)	132 (34.7)	0.19
Sleep time >9 h/day, *n* (%)	258 (68.1)	229 (60.3)	0.03

Continuous variables are expressed as median (25th–75th percentile)

Missing values: NC group: gestational age at birth *n*=1, dairy food daily *n*=1, sleep time *n*=1, physical activity *n*=4

χ^2 test (categorical variables) or Wilcoxon rank-sum test (non-normally distributed continuous variables) was used to compare the difference between the two groups

NC, naturally conceived

showed higher FBG, HOMA-IR and CIMT, lower HOMA-B, and lower serum total cholesterol and ApoA levels (ESM Table 2).

The ART group included children born after IVF and ICSI, fresh embryo transfer and frozen embryo transfer. Compared with the naturally conceived children, the children born following IVF and ICSI showed greater CIMT, higher FBG, fasting serum insulin and HOMA-IR, lower HOMA-B, and lower serum total cholesterol and ApoA levels. Similar results were found in children conceived by means of fresh and frozen embryo transfer (ESM Tables 3, 4). We further divided the children conceived by ART into four groups according to reasons for parental infertility (tubal factor, polycystic ovary syndrome [PCOS], male factor, unexplained infertility). Compared with the naturally conceived children, the children in all four of the parental infertility groups displayed greater

Table 2 Differences in metabolic variables between children conceived by ART and those conceived naturally

Multivariate model	NC group (*n*=380)	ART group (*n*=380)	Crude β (95% CI)	*p* value	Adjusted β (95% CI)	*p* value
BMI, kg/m^2	17.07±2.67	17.60±3.59	0.53 (0.08, 0.98)	0.02	−0.25 (−0.70, 0.20)[a]	0.28
Waist circumference, cm	58.8±7.5	60.8±9.4	1.93 (0.72, 3.14)	0.002	0.73 (−0.55, 2.00)[b]	0.26
FBG, mmol/l	4.57±0.55	5.06±0.37	0.49 (0.42, 0.55)	<0.001	–	–
Insulin, pmol/l	37.0 (24.4, 53.7)	48.4 (32.6, 71.5)	0.33 (0.24, 0.42)[e]	<0.001	0.28 (0.20, 0.35)[c,e]	<0.001
HOMA-IR	1.06 (0.69, 1.64)	1.57 (1.01, 2.33)	0.44 (0.34, 0.53)[e]	<0.001	0.38 (0.30, 0.46)[c,e]	<0.001
HOMA-B	105.1 (69.2, 151.1)	88.7 (64.0, 130.4)	−0.14 (−0.23, −0.06)[e]	0.001	−0.19 (−0.27, −0.11)[c,e]	<0.001
TAG, mmol/l	0.66±0.23	0.73±0.33	0.07 (0.03, 0.11)	0.001	0.03 (−0.02, 0.08)[d]	0.21
Total cholesterol, mmol/l	4.18±0.92	3.82±0.59	−0.36 (−0.47, −0.25)	<0.001	−0.42 (−0.55, −0.29)[d]	<0.001
HDL-cholesterol, mmol/l	1.60±0.38	1.58±0.33	−0.03 (−0.08, 0.02)	0.27	−0.01 (−0.06, 0.05)[d]	0.86
LDL-cholesterol, mmol/l	2.30±0.79	2.29±0.53	−0.02 (−0.11, 0.08)	0.75	−0.07 (−0.18, 0.04) [d]	0.23
ApoA, g/l	1.45±0.26	1.28±0.19	−0.17 (−0.21, −0.14)	<0.001	−0.17 (−0.21, −0.13)[d]	<0.001
ApoB, g/l	0.68±0.21	0.70±0.16	0.02 (−0.01, 0.04)	0.16	0.001 (−0.03, 0.03)[d]	0.93
CIMT, mm	0.41±0.05	0.54±0.05	0.13 (0.12, 0.13)	<0.001	–	–

Data are mean ± SD or median (25th–75th percentile)

[a] Model 1: adjusted for birthweight, maternal BMI and paternal BMI

[b] Model 2: adjusted for birthweight, soft drink intake, western-style food intake, maternal BMI and paternal BMI

[c] Model 3: adjusted for BMI of children and maternal BMI

[d] Model 4: adjusted for BMI of children, dairy intake, soft drink intake, western-style food intake, marine product intake, nut intake, duration of breastfeeding, maternal BMI, paternal age and BMI, parental education level and family history of diabetes, stroke and heart disease

[e] $\log_e$-transformed insulin, HOMA-B and HOMA-IR were considered continuous variables

ApoB, apolipoprotein B; NC, naturally conceived; TAG, triacylglycerol

CIMT, higher FBG, fasting serum insulin and HOMA-IR, and lower serum levels of total cholesterol and ApoA (ESM Table 5). We also performed a sensitivity analysis in children without a family history of diabetes and found that the differences in metabolic variables between children conceived as a result of ART and those conceived naturally remained the same (ESM Table 6).

Linear regression models were used to analyse the association between FBG and CIMT (ESM Fig. 1). Significant positive associations were found between FBG and CIMT in the total population. The ART group was then divided into two groups according to FBG level (<4.85mmol/l [25th percentile] and ≥4.85mmol/l). The CIMT in children with lower FBG levels in the ART group was still significantly higher than that in the naturally conceived children (ESM Fig. 2).

Discussion

In the present study of children at 6–10 years of age, those conceived as a result of ART were found to have higher glycometabolic and cardiovascular risk profiles than naturally conceived children, including higher fasting levels of glucose and insulin, higher HOMA-IR and lower HOMA-B, as well as greater CIMT, after controlling for birthweight, gestational age and environment factors such as diet.

Glucose homeostasis plays a key role in supporting a stable and normal growth and metabolic status for an individual. Disturbed homeostasis of glucose is thought to be the basis for metabolic disorders in later life, such as diabetes, the metabolic syndrome and cardiovascular disease, which are leading causes of death from non-communicable diseases [10]. One retrospective cohort study showed that children in the top decile for glucose, insulin and HOMA-IR were 3.28, 5.54 and 5.84 times, respectively, more likely to develop diabetes in later life than those in lower deciles [11]. Recently, it has been suggested that monitoring and intervention early in childhood may be necessary and efficient in preventing adverse metabolic outcomes later in life [12]. A recent meta-analysis indicated an increase trend in fasting insulin level, but no significant difference in fasting glucose or HOMA-IR in offspring conceived by ART [9]. It should be noted that the heterogeneity of included studies was high, which may have lowered the statistical power. According to our results, children conceived by ART showed an increased risk of hyperglycaemia and compensatory hypersecretion of insulin. Meanwhile, we observed higher HOMA-IR and lower HOMA-B values in the children conceived as a result of ART, indicating decreased insulin sensitivity and decreased secretory function of beta cells in pancreatic islets. These changes may lead to insulin-resistance-induced progressive pathophysiological processes and predispose children conceived by ART to an increased risk

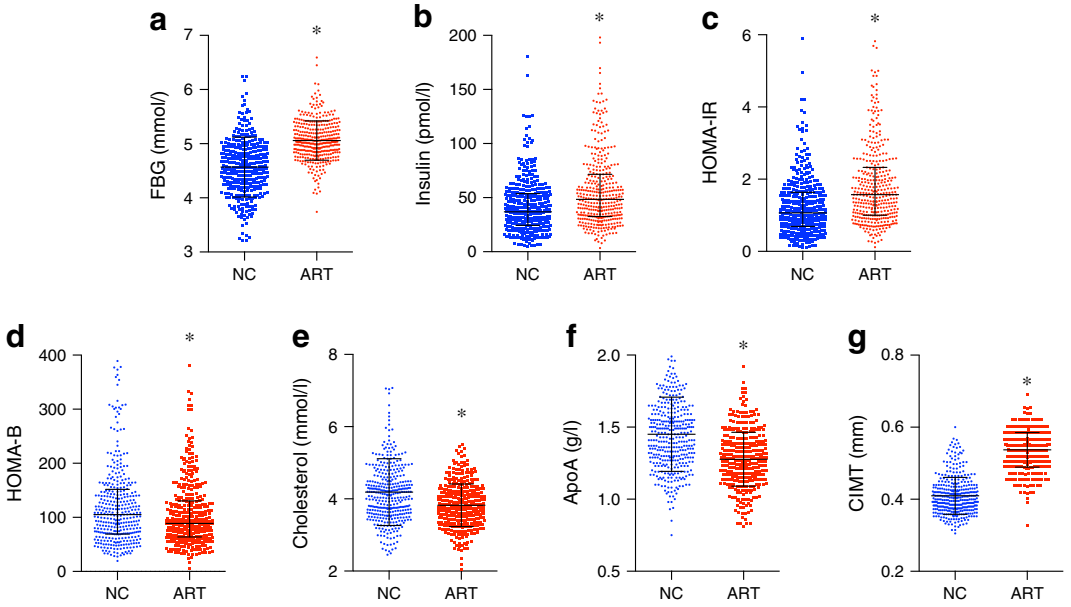

Fig. 1 Metabolic variables in children conceived by ART and those conceived naturally. FBG (**a**), fasting serum insulin (**b**), HOMA-IR (**c**), HOMA-B (**d**), total serum cholesterol (**e**), serum ApoA (**f**) and CIMT (**g**) are shown; *n*=380 (NC group); *n*=380 (ART group). Mean ± SD:FBG (mmol/l) 4.57±0.55 (NC group) vs 5.06±0.37 (ART group); total serum cholesterol (mmol/l) 4.18±0.92 vs 3.82±0.59; serum ApoA (g/l) 1.45 ±0.26 vs 1.28±0.19; CIMT (mm) 0.41±0.05 vs 0.54±0.05. Median (p25–p75): fasting serum insulin (pmol/l) 37.0 (24.4–53.7) vs 48.4 (32.6–71.5), HOMA-IR 1.06 (0.69–1.64) vs 1.57 (1.01–2.33), HOMA-B 105.1 (69.2–151.1) vs 88.7 (64.0–130.4). *p<0.05 for ART vs NC. NC, naturally conceived

of diabetes and other related complications such as cardiovascular disease.

CIMT, a proxy of arterial stiffness, is associated with premature atherosclerosis in children [13–15]. Large-scale cohort studies and high-quality meta-analyses have suggested that CIMT is an important predictor for cardiovascular disease [16, 17]. One prospective cohort study found that the adjusted HR for cardiovascular disease with a 1-SD increase in the mean CIMT was 1.13 (95% CI 1.02, 1.24) [18]. Our study revealed a greater CIMT in children born after IVF/ICSI, consistent with findings from a previous smaller study of 65 cases and 57 controls [19]. Blood glucose was found to be positively associated with CIMT both in our study and in previous reports [20]. However, after stratification by fasting glucose level, CIMT was still greater in a subgroup of children conceived by IVF/ICSI who had glucose levels comparable with those of their naturally conceived counterparts. This indicated that structural changes in vasculature in this group do indeed exist in childhood and cannot be solely explained by hyperglycaemia. Since there was evidence indicating this change might even develop early in utero [21], future studies should focus more on when and how this vasculature change occurs.

The underlying mechanisms remain unclear but possible explanations include parent-related factors such as infertility and concomitant disorders [22, 23], advanced maternal age [24, 25], non-physiological hormonal stimulation during controlled ovarian stimulation (COS) [26], and increased risk of pregnancy complications [27], as well as ART procedures such as manipulation of gametes and embryos and culture media [28]. In a previous study, birthweight and maternal age were found to be positively associated with metabolic changes in later life [29] and in our study, birthweight and maternal age were higher in the group of children conceived by ART. However, birthweight and maternal age could not fully explain the differences in metabolic profile between the children conceived by ART and those conceived naturally as the differences remained significant after adjustment for these factors. Our results also suggest that the unfavourable metabolism of children conceived by ART cannot be explained by parental infertility factors or techniques involving manipulation of gametes or embryos, as similar higher metabolic risk profiles were evident in the subgroups of children born to parents with different infertility factors, as well as after IVF, ICSI, and fresh and frozen embryo transfer. Hormonal stimulation might not be the culprit either, since the children conceived by a frozen–thaw embryo transfer (FET) method born to mothers with a normal hormone level showed similar metabolic changes to children conceived by fresh embryo transfer in our study. Collectively, future studies on the underlying mechanism need to focus more on the culture media and environmental factors during the early phase of embryo development and subsequent effects such as epigenetic reprograming [30].

Compared with previous related studies, our study has several strengths. First, the large sample size of 760 individuals provided a more confident conclusion based on greater statistical power. Furthermore, the large sample size also

allowed for subgroup analyses by which to explore potential underlying mechanisms. Second, the study's matched design and single laboratory measurements increased the homogeneity of the data and therefore decreased the confounding effects to some extent. Third, our study focused on children aged 6–10 years, meaning that the children were old enough to be evaluated for metabolic function but not too old to be impacted by puberty development.

Nonetheless, several limitations are worth mentioning. First, the comparability of the children conceived as a result of ART and those conceived naturally may be compromised to some degree. To overcome this potential deficiency, we tried to control for as much information as possible but residual confounding may still be possible. Second, half of the women in the ART group were lost to follow-up. Although there was no significant difference in most of the basic characteristics between the enrolled and withdrawn ART population, the maternal age was lower in the study group. It has been reported that older maternal age is related to higher fasting glucose concentration [31]. We matched our ART and natural conception groups by maternal age (±2 years), thus this bias may have little impact on the results. Third, although naturally conceived children were matched by maternal age at birth to the children conceived by ART, the maternal age of the ART group was higher. The percentage of women with a family history of diabetes was also higher in the ART group than in the natural conception group (25.3% vs 19.2%, p=0.04), and maternal BMI was higher. Thus, we adjusted for maternal age, maternal BMI and family history of diabetes in our model. However, residual confounding could not be totally ruled out. We further divided the ART group into four groups according to reasons for infertility (tubal factor, PCOS, male factor, unexplained infertility). Compared with the normally conceived children, those conceived by ART in the absence of PCOS also displayed greater CIMT, higher FBG and fasting serum insulin levels, higher HOMA-IR and lower HOMA-B. In addition, we analysed the difference in metabolic variables between ART and naturally conceived children in those without family history of diabetes; the results remained the same. This suggests that ART may exert an adverse effect on offspring, even in women without PCOS and diabetes. However, we still cannot totally discount the effect of parental factors that may potentially influence the genetic propensity to cardiometabolic disease, or shared environment. Future study is required to distinguish the accurate contribution of ART and parental diseases on metabolic indicators in children. Fourth, energy intake is an important confounding factor for evaluation of metabolism. Although we took diet composition into consideration, this was not as accurate as calculation of energy intake in kJ or kcal. However, it is notable that our findings showed that children conceived after ART had similar levels of LDL-cholesterol, HDL-cholesterol and triacylglycerol, and even lower levels of total cholesterol and ApoA, when compared with naturally conceived children. These favourable lipid profiles indicated that the premature artery stiffness may not be due to diet-related dyslipidaemia. This previously unreported association between ART and total cholesterol requires replication. Finally, although we have adjusted for many covariates, there may be other potential confounders, such as parity, gestational diabetes mellitus and gestational weight gain, that were not included. Our results support our hypothesis that offspring born following ART had a higher risk of metabolism dysfunction in childhood. However, further prospective cohort studies with a better study design, such as detailed records of reproductive history and pregnancy complications, may be needed to confirm or refute our findings.

In conclusion, offspring born after ART had higher risk of metabolism dysfunction in childhood manifested as higher glucose level, decreased insulin sensitivity and secretion, and arterial stiffness. The underlying mechanism is still unknown but exposure to in vitro environment during the early phase of embryo development might play a critical role. Increased metabolic and cardiovascular risk profiles in children conceived by ART is of importance at the individual level as well as for the whole of society. Continuous monitoring and early intervention should be fully considered for this group of the population.

Data availability The data are available from the corresponding author on reasonable request.

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Contribution statement LC, BX and Z-JC were involved in the design of the study. WZ conducted the data analysis. All authors were involved in the recruitment of participants and blood sample collection. LC and WZ completed the first draft of the manuscript. All authors were involved in critical revision of the manuscript. All authors read and approved the final manuscript. LC, WZ and Z-J C guarantee this work and take responsibility for the integrity of the data.

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